DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL ANTITUBERCULAR AGENTS

A dissertation submitted to

The Tamilnadu Dr. M.G.R Medical University

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IN

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Submitted by

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DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

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CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN, SYNTHESIS, CHARACTRIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL ANTITUBERCULAR AGENTS"** submitted by the candidate bearing the **Reg. No. 261215705** in partial fulfillment of the requirements for the award of the degree of **MASTER OF PHARMACY in PHARMACEUTICAL CHEMISTRY** by **The Tamilnadu Dr. M.G.R Medical University** is a bonafide work done by him during the academic year 2013-2014 at the **Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-3**.

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DEDICATED TO ALMIGHTY, MY FAMILY, TEACHERS AND FRIENDS

CONTENTS

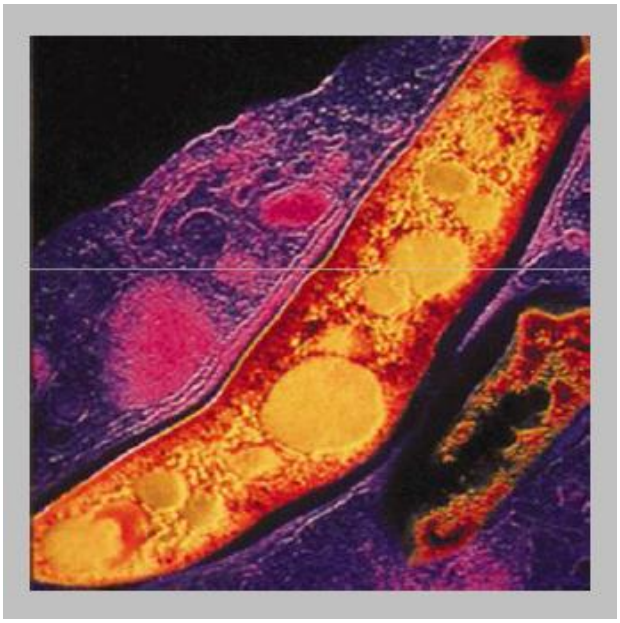
LIST OF ABBREVIATIONS

ABBREVIATION	EXPANSION
Log P	Partition co-efficient
Log D	Diffusion co-efficient
Da	Daltons
MmaA2	Alpha mycolic acid
TLC	Thin Layer Chromatography
IR	Infrared
NMR	Nuclear magnetic resonance spectroscopy
3D	Three dimensions
MABA	Microplate Alamar Blue Assay
MIC	Minimum inhibitory concentration
GLIDE	Grid Based Ligand Docking Energitics
PSA	Polar Surface Area
TPSA	Total Polar Surface Area
CoMFA	Comparative molecular field analysis
CoMSIA	Comparative molecular similarity index analysis
OSIRIS	Optical spectroscopic and infrared remote imaging system

1.1 TUBERCULOSIS [7, 8, 9, 11]

Tuberculosis (TB) is becoming a serious problem worldwide, and is responsible for at least three million deaths annually, as a life is lost to TB every 15 seconds. Factors involved in the resurgence of TB. Tuberculosis (TB) is a chronic bacterial infection, spread through the air, and caused by Mycobacterium tuberculosis (MTB) which is an aerobic bacilli belonging to the Mycobacteriaceae family. It was first identified in 1882 by Robert Koch. It mainly attacks lungs, although it can affect other organs as well.

Fig.1



NEED FOR FOCUS ON TUBERCULOSIS DISEASE:

- Approximately 50% of the India's population is reported to be tuberculin test positive
- This led to the declaration of TB as a global emergency by WHO 1993. The regeneration of TB is closely linked to the emergence of HIV and total deficiency of the immune system. The WHO estimate is that up to 50 million persons worldwide are infected with drug resistant strains of TB. On addition, 300,000 new cases of MDR-TB are diagnosed around the world each year and 79% of the MDR-TB cases now show resistance to three or more of the commonly used drugs.
- More than 300,000 Indian children leave school every year, as they need to care for their parents who have TB .many end up getting it.
- More than 100,000 Indian women suffering from TB are rejected by their families

- The HIV epidemic will increase tuberculosis cases by at least 200,000 each year in India, if HIV spreads more rapidly; tuberculosis may become uncontrollable for a generation. Despite enormous efforts, no new drug has been introduced in the market for the past 40 years.
- The re-emergence of tuberculosis (TB) as a global health problem over the past few decades, accompanied by the rise of drug-resistant strains of Mycobacterium tuberculosis, emphasizes the need for discovery of new therapeutic drugs against this disease.
- The emerging serious problem both in the terms of TB control and clinical management
- Prompted us to search for new molecule as anti-tubercular agents.

Patterns of Infection

There are two major patterns of disease with TB:

- Primary tuberculosis: seen as an initial infection, usually in children. The initial focus of infection is a small sub pleural granuloma accompanied by granulomatous hilar lymph node infection. Together, these make up the Ghon complex. In nearly all cases, these granulomas resolve and there is no further spread of the infection.
- Secondary tuberculosis: seen mostly in adults as a reactivation of previous infection (or reinfection), particularly when health status declines. The granulomatous inflammation is much more florid and widespread. Typically, the upper lung lobes are most affected, and cavitation can occur.

When resistance to infection is particularly poor, a "miliary" pattern of spread can occur in which there are a myriad of small millet seed (1-3 mm) sized granulomas, either in lung or in other organs.

Dissemination of tuberculosis outside of lungs can lead to the appearance of a number of uncommon findings with characteristic patterns:

Skeletal Tuberculosis: Tuberculosis osteomyelitis involves mainly the thoracic and lumbar vertebrae (known as Pott's disease) followed by knee and hip. There is extensive necrosis and bony destruction with compressed fractures (with kyphosis) and extension to soft tissues, including psoas "cold" abscess.

Genital Tract Tuberculosis: Tuberculosis salpingitis and endometritis result from dissemination of tuberculosis to the fallopian tube that leads to granulomatous salpingitis, which can drain into the endometrial cavity and cause a granulomatous endometritis with irregular menstrual bleeding and infertility. In the male, tuberculosis involves prostate and epididymis most often with non-tender induration and infertility.

Urinary Tract Tuberculosis: A "sterile pyuria" with WBC's present in urine but a negative routine bacterial culture may suggest the diagnosis of renal tuberculosis. Progressive destruction of renal parenchyma occurs if not treated. Drainage to the ureters can lead to inflammation with ureteral stricture.

CNS Tuberculosis: A meningeal pattern of spread can occur, and the cerebrospinal fluid typically shows a high protein, low glucose, and lymphocytosis. The base of the brain is often involved, so that various cranial nerve signs may be present. Rarely, a solitary granuloma, or "tuberculoma", may form and manifest with seizures.

Gastrointestinal Tuberculosis: This is uncommon today because routine pasteurization of milk has eliminated Mycobacterium bovis infections. However, M. tuberculosis organisms coughed up in sputum may be swallowed into the GI tract. The classic lesions are circumferential ulcerations with stricture of the small intestine. There is a predilection for ileocecal involvement because of the abundant lymphoid tissue and slower rate of passage of luminal contents.

Adrenal Tuberculosis: Spread of tuberculosis to adrenals is usually bilateral, so that both adrenals are markedly enlarged. Destruction of cortex leads to Addison's disease.

Scrofula: Tuberculosis lymphadenitis of the cervical nodes may produce a mass of firm, matted nodes just under the mandible. There can be chronic draining fistulous tracts to overlying skin. This complication may appear in children, and Mycobacterium scrofulaceum may be cultured.

Cardiac Tuberculosis: The pericardium is the usual site for tuberculosis infection of heart. The result is a granulomatous pericarditis that can be haemorrhagic. If extensive and chronic, there can be fibrosis with calcification, leading to a constrictive pericarditis.

The following images illustrate gross pathologic findings with tuberculosis:

Microscopic Findings

Microscopically, the inflammation produced with TB infection is granulomatous, with epithelioid macrophages and Langhans giant cells along with lymphocytes, plasma cells, maybe a few PMN's, fibroblasts with collagen, and characteristic gaseous necrosis in the center. The inflammatory response is mediated by a type IV hypersensitivity reaction. This can be utilized as a basis for diagnosis by a TB skin test. An acid fast stain (Ziehl-Neelsen or Kinyoun's acid fast stains) will show the organisms as slender red rods. An auramine stain of the organisms as viewed under fluorescence microscopy will be easier to screen and more organisms will be apparent. The most common specimen screened is sputum, but the histologic stains can also be performed on tissues or other body fluids. Culture of sputum or tissues or other body fluids can be done to determine drug sensitivities.

Tuberculin Skin Testing

Skin testing for tuberculosis is useful in countries where the incidence of tuberculosis is low, and the health care system works well to detect and treat new cases. In countries where BCG vaccination has been widely used, the TB skin test is not useful, because persons vaccinated with BCG will have a positive skin test.

The TB skin test is based upon the type 4 hypersensitivity reaction. If a previous TB infection has occurred, then there are sensitized lymphocytes that can react to another encounter with antigens from TB organisms. For the TB skin test, a measured amount (the intermediate strength of 5 tuberculin units, used in North America) of tuberculin purified protein derivative (PPD) is injected intracutaneously to form a small wheal, typically on the forearm. In 48 to 72 hours, a positive reaction is marked by an area of red induration that can be measured by gentle palpation (redness from itching and scratching doesn't count). Reactions over 10 mm in size are considered positive in non-immunocompromised persons.

Repeated testing may increase the size of the reaction (induration), but repeated TB skin testing will not lead to a positive test in a person not infected by TB. Anergy, or absence of PPD reactivity in persons infected with TB, can occur in

immunocompromised persons, or it may even occur in persons newly infected with TB, or in persons with miliary TB.

1.2. MEDICINAL CHEMISTRY

Medicinal chemistry is a discipiline at the intersection of chemistry and pharmacology and involves the drug discovery process.

The drug discovery process involves designing, synthesizing, characterization and evaluation of new chemical entities, suitable for therapeutic use.it also includes the study of existing drugs, their biological properties, and their quantitative structure activity relationship (QSAR)

PROCESS IN DRUG DISCOVERY

DISCOVERY [23]

Discovery is the identification of novel active compounds, often called "hits", which are typically found by screening many compounds (compound library) for the desired biological properties. While a number of approaches toward the identification of hits exist, the most successful of techniques relies on chemical and biological intuition developed through years of rigorous chemical –biological training. Other sources of hits can come from natural sources, such as plants, animals, or fungi. Hits may originate also from synthetic chemical libraries, such as those created through combinatorial chemistry or historic chemical compound collections that are tested enmasse against the biological target in question.

OPTIMIZATION:

Another step in drug discovery involves further chemical modifications in order to improve the biological and physiochemical properties of a given candidate compound library. Chemical modifications can improve the recognition and binding geometries (Pharmacophore) of the candidate compounds, their affinities and pharmacokinetics, or indeed their reactivity and stability during their metabolic degradation .which exhibit the most potency, most selectivity, best pharmacokinetics and least toxicity.

QSAR involves mainly physical, chemistry and molecular docking tools (COMFA and COMSIA), that leads to tabulated data and first and second order equations. There are many theories, the most relevant being Hansch, analysis that involves Hammett electronic parameters, steric parameters and logP (lipophilicity) parameters.

1.3. DRUG DESIGN [5]

Drug design, also sometimes referred to as rational drug design, is the inventive process of finding new medications based on the knowledge of the biological target. The drug is most commonly an organic small molecule which activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves design of small molecules that are complementary in shape and charge to the biomolecular target to which they interact and therefore will bind to it. Drug design frequently but not necessarily relies on computer modelling techniques. This type of modelling is often referred to as computer-aided drug design (CADD).

1.3.1 TYPES

There are two major types of drug design. The first is referred to as ligand based drug design and the second, structure-based drug design.

LIGAND BASED

Ligand –based drug design (or indirect drug design) relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a Pharmacophore model which defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.in other words, a model of the biological target may be build based on the knowledge of what binds to it and this model in turn may be used to design new molecular entities that interact with the target. Alternatively , a quantitative structure –activity relationship (QSAR) in which a correlation between calculate properties of molecules and their experimentally determined biological activity may be derived .these QSAR relationships in turn may be used to predict the activity of new analogues.

STRUCTURE BASED: Structure - based drug design (or direct drug design) relies on knowledge of the three dimensional structure of the biological target obtained

through methods such as x –ray crystallography or NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. Alternatively various automated computational procedures may be used to suggest new drug candidates

1.3.2 PRESENT AND FUTURE

Today, computer-aided drug design and screening methods impacts the efforts impact the efforts of all pharmaceutical companies.as the computational technologies advance, the role they play in improving the efficiency of the drug discovery process will become increasingly important. In addition, as the body of structural information on potential therapeutic targets dramatically expands, which is expected to happen in the next few years, it will drive the development of the computational methodology. Greater automation, faster algorithms and improved information management techniques will be required to handle sheer volume of target related information that will need to be processed. On a genomic scale, instead of looking at individual targets, families of related targets will be studied. The information available on ligand binding to these families will be vastly expanded. The job of the molecular modeller will be to effectively mine this data as well as translate the available structural information into a form directly usable by the bench chemist. This mission will ultimately cause a greater interface of bio and chemo informatics, leading to improved structural and functional genomics knowledge.

1.3.3 DOCKING [21]

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of small molecule. Hence docking plays an important role in the rotational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking. The quality of any docking results depends on reasonable starting structures for both the protein and the ligand. The protein and the ligand structures need to be preparing to achieve the best docking results.

PROTEIN PREPARATION:

A typical PDB (protein data bank) structure file consists of heavy atoms and contains water, cofactors, and metal ions and can be multimeric. The structure generally has information on bond orders, topologies, or formal atomic charges. Terminal amide groups can also be misaligned, because the x-ray structure analysis cannot usually between O and NH2.Ionisation and tautomeric states are generally unassigned.

The following processes are necessary to make protein to perfect structure for docking study:

1. Assign ionisation and tautomer states of protein properly. (Side chains are reoriented when necessary and steric clashes are relieved.)

2. Delete all water molecules (except those coordinated to metals, if water molecules are kept, hydrogens will be added to them.)

3. Adjust the protonation of the protein, which is crucial when the receptor site is metalloproteinase

4. Finally minimize the protein to reorient side chain hydroxyl groups and alleviate potential steric clashes present in the PDB structure.

LIGAND PREPARATION

To give the best results, the structures that are docked must be good representations of the actual ligand structures as they would appear in a protein –ligand complex. Most of the docking tools only modify the torsional internal coordinates of the ligand during docking, so the rest of the geometric parameters must be optimized beforehand. This means that the structures supplied to docking tool must meet the following conditions:

- They must be three-dimensional (3d)
- They must have realistic bond lengths and bond angles
- They must each consist of a single molecule that has no covalent bonds to the receptor, with no accompanying fragments, such as counter ions and solvent molecules

- They must have all their hydrogens (filled valences)
- They must have an appropriate protonation state for physiological pH values

ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION (ADME) ANALYSIS [29]

DRUG DISCOVERY and development are expensive and time –consuming processes. Recognition by the pharmaceutical industry that undesirable absorption ,distribution, metabolism and excretion(ADME) properties of new drug candidates are the cause of many clinical phase drug development failures has resulted in a paradigm shift to identify such problems early in the drug discovery process. Thus *in vitro* approaches are now widely used to investigate the ADME properties of new chemical entities and more recently, computational (in *silico*) modelling has been investigated as a tool to optimise selection of the most suitable drug candidates for development.

The objective of in silico modelling tools is for predicting these properties to serve aim key aims. First, at the design stage of new compounds and compound libraries so as to reduce the risk of late –stage attrition; and second , to optimize the screening and testing by looking at only the most promising compounds.

Drug like properties

The properties which can differentiate drugs from other chemicals can be considered as a drug like properties. The crucial properties that should be considered for compounds with oral delivery (Lipinski's rule - of - five) includes molecular mass <500 Daltons(Da), calculated octanol/water partition coefficient(CLOGP)<5 number of hydrogen –bond donors<5 and number of hydrogen bond acceptors<10. These properties are than typically used to construct predictive ADME models and form the basis for what has been called property-based design.

A deeper understanding of the relationships between important ADME parameters and molecular structure and properties has been used to develop in silico models that allow the early estimation of several ADME properties. Among other important issues, prediction of properties that provide information about dose size and dose frequency such as oral absorption, bioavailability, brain penetration, clearance (for exposure) and volume of distribution (for frequency) also needed.

Prediction of ADME and related properties [45]

Absorption

For a compound crossing a membrane by purely passive diffusion, a reasonable permeability estimate can be made using single molecular properties, such as log D (diffusion co efficient) or hydrogen –bonding capacity. The simplest Insilco models for estimating absorption are based on a single descriptor, Such as logP (partition coefficient) or log D, or polar surface area, which is a descriptor of hydrogen-bonding potential. Different multivariate approaches such as multiple liner regressions, partial least squares and artificial neural networks, have been used to develop quantitative structure-human-intestinal-absorption relationships.

Bioavailability

Important properties for determining permeability seem to be the size of the molecule, as well as its capacity to make hydrogen bonds, its overall lipophilicity and possibly its shape and flexibility.

Blood –Brain Barrier penetration (BBB)

Drugs that act in the CNS need to cross the blood –brain barrier (BBB) to reach their molecular target.by contrast, for drugs with a peripheral target, little or no BBB penetration might be required in order to avoid CNS side effects. Rule –of –five like recommendations regarding the molecular parameters that contribute to the ability of molecules to cross the BBB have been made to aid BBB-penetration predictions; for example molecules with a molecular mass of <450Da or with polar surface area (PSA) <100A0 are more likely to penetrate the BBB.

Dermal and ocular penetration

The existing transdermal models are typically a function of the octanol/water partition coefficient and dermas that have been associated with aqueous solubility, including hydrogen – bonding parameters, molecular weight and molecular flexibility. Commercial models for the prediction of solute –permeation rates through the skin are available, for example Qikrop and DermWin programs.

METABOLISM

In silico approaches to predicting metabolism can be divided into QSAR and three –dimensional-QSAR studies, protein and Pharmacophore models and predictive databases. Some of the first –generation predictive –metabolism tools currently require considerable input from a computational chemist, whereas others can be used as rapid filters for the screening of virtual libraries. Perhaps the most intellectually satisfying molecular modeming studies are those based on the crystal structure of the metabolizing enzymes. Several approaches that use databases to predict metabolism are available. Ultimately, such programs might be linked to computer-aided toxicity prediction on the basis of quantitative structure-toxicity relationships and expert systems for toxicity evaluation.

In silico PREDICTION OF TOXICITY ISSUES

Toxicity is responsible for many compounds failing to reach the market and for the withdrawal of a significant number of compounds from the market once they have been approved. It has been estimated that -20-40% of drug failures in investigational drug development can be attributed to toxicity concerns. The existing commercially available in silico tools for forecasting potential toxicity issues can be roughly classified into two groups. The first approach uses expert systems that derive models on the basis of abstracting and codifying knowledge from human experts and the scientific literature. The second approach relies primarily on the generation of descriptors of chemical structure and statistical analysis of the relationships between these descriptors and the toxicological end –point.

The primary emphasis of the current software packages is carcinogenicity and mutagenicity, although some packages do also include models and /or knowledge bases for other end –points, such as teratogenicity, irritation, sensitization, immunotoxicology and neurotoxicity .there is currently an unmet need for in silico predictive toxicology software for other end –points important in drug development, such as QT prolongation hepatotoxicity and phospholipidosis.

BINDING SITE ANALYSIS

Understanding the structure and function of protein binding sites is the cornerstone of structure –based drug design .developing this understanding requires knowledge of both the location and physical properties of the binding site .in addition ,the identification of small –molecule binding sites as modulators of protein-protein interactions is of increasing interest furthermore, even when a validated binding site has been identified ,it is often important to find additional potential binding sites where appropriate targeting could result in different biological effects or new classes of compounds .when the binding site is not known from a 3-D structure or from other experimental data, computational methods can be employed to suggest likely locations. When the location of the primary binding site is known, medicinal chemistry efforts to design better ligands can profit from a better understanding of the degree to which known ligands are ,or fail to be ,complementary to the receptor as well as from a critical assessment of the degree to which the occupancy of accessible but unexplored regions by appropriate ligand functionality can be expected to promote binding or could be used to improve the physical properties of the ligand without lessening its binding affinity. Such assessments can assist in the evaluation and optimization both of known binding molecules and of virtual screening hits.it is also important to understand the potential drugs ability of the site.

SCORING METHODS

Scoring of docked poses is still regarded as one of the major challenges in the field of molecular docking. The purpose of the scoring procedure is the identification of the correct binding pose by its lowest energy value, and the ranking of protein –ligand complexes according to their binding affinities. Scoring functions can be divided in empirical scoring functions, scoring functions derived from force fields, and knowledge –based scoring functions. Scoring functions derived from force fields handle the ligand binding prediction with the use of potential energies (non –bonded interaction terms) and sometimes in combination with solvation's and entropy contributions knowledge- based scoring functions are based on atom pair potentials derived from structural databases forces and potentials are collected from known protein –ligand complexes to get a score for their binding affinities.

1.4 ENZYME PROFILE [30]

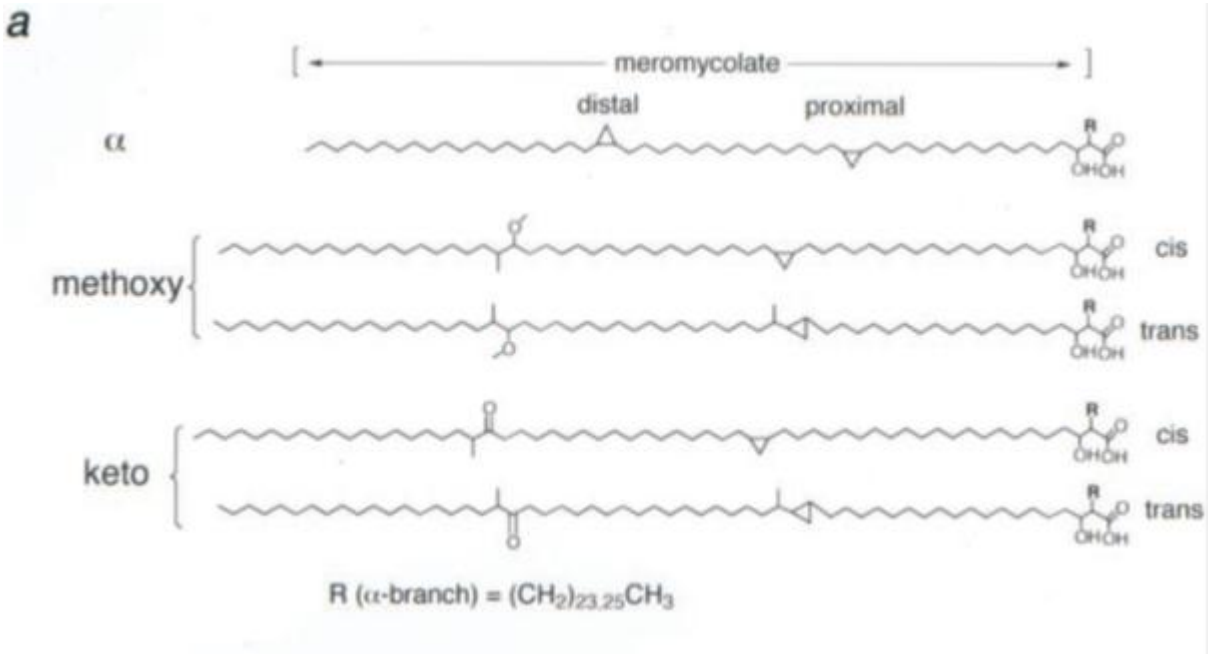
MmaA2 is required for introduction of the distal cyclopropane ring in the formation of meroacids. Analysis of a *mmaA2* deletion mutant of *M. tuberculosis* revealed that _-mycolic acid lacks a distal cyclopropane group and instead contains a *cis* unsaturation. Thus, *mmaA2* is required for the distal cyclopropane modification of _-mycolic acid

Functional category: lipid metabolism

Targeting Cell Wall Synthesis [36, 39]

Mycolic acids, which are key components of the mycobacterial cell wall, are alpha-alkyl, beta-hydroxy fatty acids, with a species-dependent saturated "short" arm of 20-26 carbon atoms and a "long" mero mycolic acid arm of 50-60 carbon atoms. The latter arm is functionalized at regular intervals by cyclopropyl, alpha-methyl ketone, or alpha-methyl methyl ethers groups. The mycolic acid biosynthetic pathway has been proposed to involve five distinct stages: (i) synthesis of C20 to C26 straight-chain saturated fatty acids to provide the alpha-alkyl branch; (ii) synthesis of the mero mycolic acid chain to provide the main carbon backbone, (iii) modification of this backbone to introduce other functional groups; (iv) the final Claisen-type condensation step followed by reduction; and (v) various mycolyltransferase processes to cellular lipids. The drugs shown to inhibit mycolic acid biosynthesis are isoniazid, ethionamide, isoxyl, thiolactomycin, and triclosan. In addition, pyrazinamide was shown to inhibit fatty acid synthase type I which, in turn, provides precursors for fatty acid elongation to long-chain mycolic acids by fatty acid synthase II.

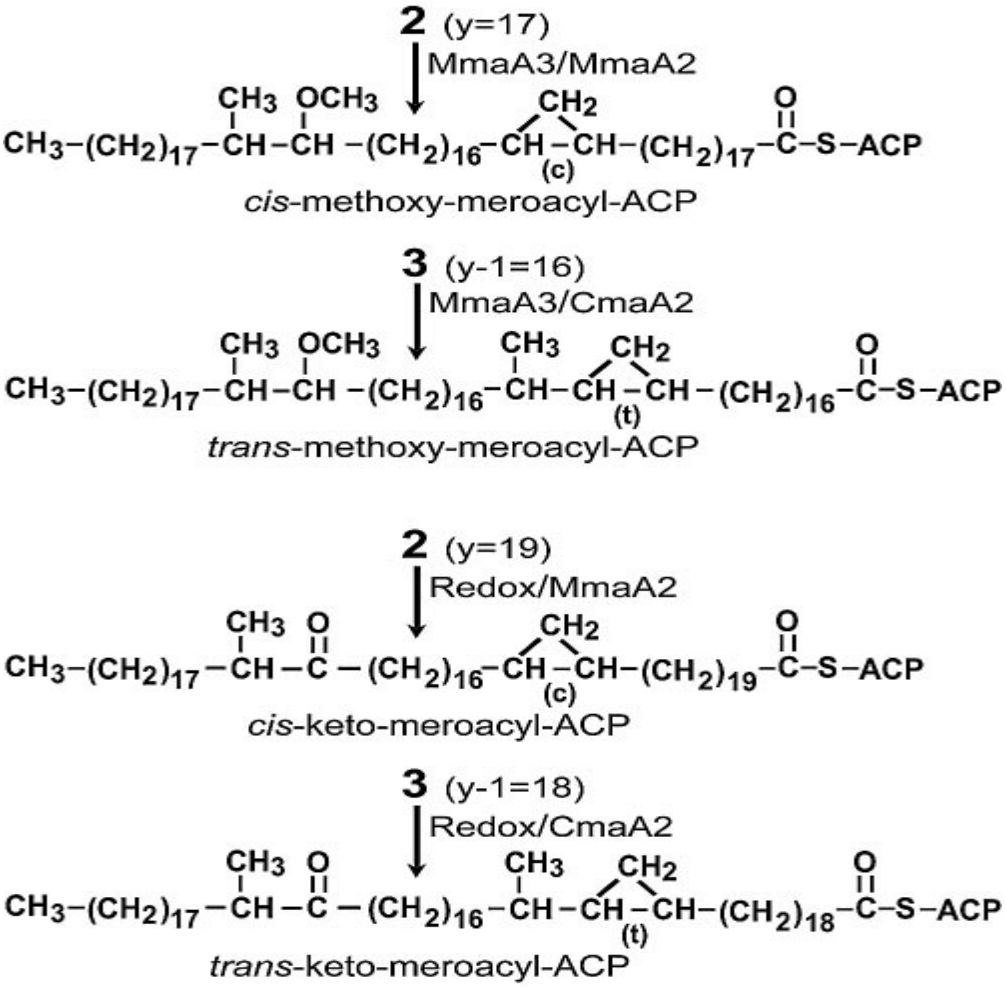
Structures of Mycolic Acid Cyclopropane Synthases [2, 3, 21] **Fig.2**



Mycolates contain cyclopropane rings at both the distal and the proximal positions. Methoxymycolates and ketomycolates have *cis* or *Tran's* cyclopropane rings at the proximal position and the oxygenated functional group at the distal position. (i) The cyclopropane rings of mycolic acids in *M. tuberculosis* contribute to the structural integrity of the cell wall complex. Moreover, the cyclopropane rings protect the bacillus from oxidative stress. Thus, the structure of mycolic acids determines the degree of protection that *M. tuberculosis* receives against the hostile environment of the host. (ii) Deletion of the proximal cyclopropane ring of mycolate or deletion of the methoxy- and keto-mycolates leads to a significant_attenuation of growth of the two mutants in the mouse model of infection. Deletion of keto-mycolates in *M.tuberculosis* leads to restricted growth of this mutant in macrophages. Thus, the distribution and fine structure of mycolic acids determine the virulence of *M tuberculosis.* It can be concluded that mycolic acids are very important components of the pathogenic *M. tuberculosis.* (iii) Mycolic acid synthesis is the target of well-known antituberculosis drugs, i.e., isoniazid, ethionamide, and thiocarlide. This suggests that all reactions on the pathway to synthesis and processing of mycolic acids are viable targets for new drug discovery (target validation) and to find new and more effective drugs against multiple-drug-resistant strains of *M. tuberculosis*.

The cell wall contains a very high proportion of lipid in the form of mycolic acid. Mycolic acids are long-chain, two-branched, 3-hydroxy fatty acids that range in size in mycobacteria from '60 to 80 carbons and contain a variety of functional groups on the long (mero) chain . These functional groups are either nonpolar moieties, such as olefins, methyl branches, and Cyclopropane, or polar moieties, such as ketones, methoxy groups, epoxides, and esters. The distribution of functional groups among species is distinct and useful for taxonomic identification of species. In *M. tuberculosis*, three distinct mycolate species are produced, a mycolates containing two *cis*-cyclopropane rings and a methoxy and keto series, each of which contains a *cis*- or *trans*-cyclopropane in the proximal position, in addition to the distal oxygen function with an adjacent methyl branch. In *M. tuberculosis* and related organisms, methoxy- and ketomycolates are generally of lower abundance than a mycolates, although the exact ratios are dependent upon growth conditions.

Fig.3



Methyltransferases and oxidation-reduction (redox) to form the methoxy group of methoxy-meroacids and the Oxo group of keto-meroacids on the pathway to synthesis of oxygenated meroacids. MmaA2 introduces the methyl group on the secondary alcohol, MmaA3 introduces the proximal *cis*-cyclopropane ring, and CmaA2 introduces the proximal *trans*-cyclopropane ring. Redox is the proposed oxidation-reduction system that converts the secondary alcohol to an Oxo group. T and c, *Trans* and *cis* isomers, respectively.

The Field of Heterocyclic Chemistry

For centuries ago, heterocyclic have contributed their presence in the field of research and development in organic chemistry.as a result, millions of heterocyclic compounds found to exist, synthesized having special properties with biological importance. Recently, analysis of organic compounds as of June 2007, there were 24,282,284, compounds were registered in chemical abstracts containing cyclic structures with heterocyclic systems making up of many compounds. Among various heterocyclic compounds, we have chosen pyridine based compound namely, acridine.

ACRIDINE

Acridine is structurally related to anthracene with one of the central CH groups is replaced by nitrogen. Acridine, a colourless solid, was first isolated from coal tar. It is a raw material used for the production of dyes and some valuable drugs. Many acridines, such as proflavine, also have antiseptic properties. Acridine and related derivatives bind to DNA and RNA due to their abilities to intercalate. Acridine orange (3,6-dimethylaminoacridine)is anucleicacid-selective etachromatic stain useful for cell cycle determination. Acridarsine is formally derived from acridine by replacing the nitrogen atom with one of arsenic, and acridophosphine by replacing it with one of phosphorus.

1.5 SCHIFF BASE [1, 16, 26]

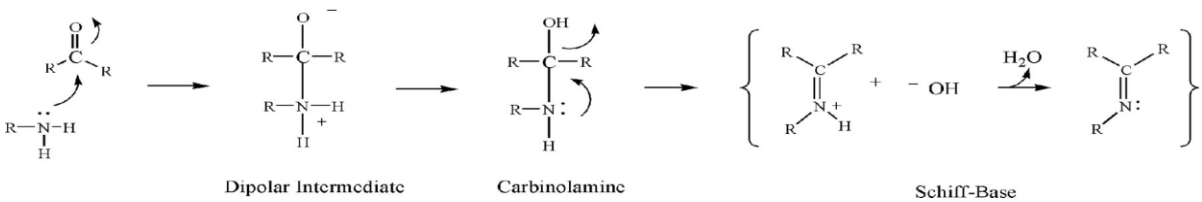
Schiff bases are condensation products of primary amines with carbonyl compounds and they were first reported by Schiff in 1864. The common structural feature of these compounds is the azomethine group with a general formula RHC=N-R1, where R and R1 are alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted. These compounds are also known as anils, imines or azomethines. Several studies showed that the presence of a lone pair of electrons in a sp2 hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical and biological importance. Because of the relative easiness of preparation, synthetic flexibility, and the special property of C=N group, Schiff bases are generally excellent chelating agents, especially when a functional group like –OH or –SH is present close to the azomethine group so as to form a five or six membered ring with the metal ion. Versatility of Schiff base ligands and biological, analytical and industrial applications of their complexes make further investigations in this area highly desirable.

Schiff bases have been known since 1864 when Hugo Schiff reported the condensation of primary Amines with carbonyl compounds. Nowadays, the research field dealing with Schiff base coordination chemistry has expanded enormously. The importance of Schiff base complexes for bioinorganic chemistry, biomedical applications, supramolecular chemistry, catalysis and material science, separation and encapsulation processes, and formation of compounds with unusual properties and structures has been well recognized and reviewed.

Reaction Mechanism

Schiff bases are synthesized from an aromatic amine and a carbonyl compound by nucleophilic addition forming a hemiaminal, followed by a dehydration to generate imines.

Fig.4



LITERATURE REVIEW

Literature review on Tuberculosis research

- **Williams, B.G *et al.*, (2010)** studied about the "The Population Dynamics and Control of Tuberculosis."

- **De Souza MVN, et al., (2006)** Current status and future prospects for new therapies for pulmonary tuberculosis.

- **Duncan K, et al., (2004)** Prospects for new antitubercular drugs.

- **Cohn, D. L *et al.*, (1997)** reported a "Drug-resistant tuberculosis: review of the worldwide situation and the WHO/IUATLD global surveillance project
- **Glickman SW *et al.*, (2006)** A Portfolio model of drug development for tuberculosis.

Literature review on Target of interest- Alpha methoxy Mycolic acid inhibitor

- **Asselineau.J *et al.*, (1950)** reported "Structure of the mycolic acids of mycobacteria''
- **Barry.C.E., *et al.*, (1998)** reported "Mycolic acids: structure, biosynthesis and physiological functions.
- **Ying Yuan *et al.*, (1996)** reported "A common mechanism for the biosynthesis of methoxy and cyclopropyl mycolic acids in *Mycobacterium tuberculosis.*
- **Santos.DS *et al.*, (2002)** reported the Drugs that inhibit mycolic acid biosynthesis in Mycobacterium tuberculosis.
- **Michael S. Glickman *et al.*, (2002)** Acid cyclopropane synthase of the alpha mycolic tuberculosis encodes the distal the mmaA2 gene of Mycobacterium
- **Gurdyal S. Besra *et al.,(* 2005,)** studied a '' Pathway to Synthesis and Processing of Mycolic Acids in Mycobacterium tuberculosis'

Literature review on drug design

- **Guner Osman.F (2000)** reported a Pharmacophore Perception, Development, and use in Drug Design.
- **Schnecke,V *et al*.,.(2000)** wrote a book on Perfect Drug Discov.
- **Lipinski CA *et al*., (2001)** reported a "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings".
- **Oprea TI (2001)** wrote a book on "Is there a difference between leads and drugs? A historical perspective".
- **Tarbit MH.*et al*., (2002)** reported "The emerging importance of predictive ADME simulation in drug discovery''
- **Madsen *et al*., (2002)** wrote a book on Textbook of Drug Design and Discovery.
- **Kitchen DB, *et al*., (2004)** reported a "Docking and scoring in virtual screening for drug discovery: methods and applications".
- **Lipinski CA (2004)** reported a "Lead- and drug-like compounds: the rule-of-five revolution".
- **P. LALITHA *et al*.,(2010)** reported a Calculation of molecular lipophilicity and drug likeness for few heterocycles.

- **Cohen.NClaude (1996)** wrote a book on Guidebook on Molecular Modelling in Drug Design.

Literature review on Biological screening

- **Scott G.Franzblau *et al*., (1998)** studied the Rapid, Low-Technology MIC Determination with Clinical *Mycobacterium tuberculosis* Isolates by Using the Microplate Alamar Blue Assay.

- **Bornman, D.L *et al*., (2001)** reported an Assessment of a simple, non –toxic Alamar Blue cell survival assay to monitor tomato cell viability.

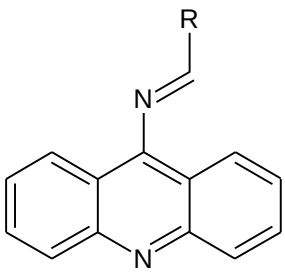
- **Maria C. S. *et al*., (2007)** reported an Evaluation of anti-Tubercular activity of nicotinic and isoniazid analogues
- **Sephra.N.Rampersad *et al.*,** reported a Multiple applications of Alamar Blue as an Indicator of Metabolic Function and cellular Health in Cell Viability Bioassays.

Literature review for Spectroscopy

- **P.S.Kalsi** wrote a book on Spectroscopy of Organic compounds.
- **D.Kealey *et al.*,** wrote a book on Instant notes Analytical chemistry.
- **Gurdeep R.Chatwal (2005)** wrote a book on, Instrumental methods of chemical analysis.

Literature review on Schiff base

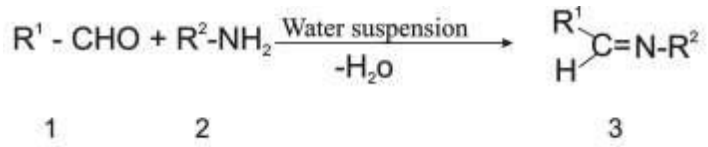
- **Denny.W.A *et al*., (1982)** studied about the "Potential antitumor agents. 36. Quantitative relationships between experimental antitumor activity, toxicity, and structure for the general class of 9-anilinoacridine antitumor agents.
- **Rivera et *al., (*2007)** studied the complexes of Pd(11)and Pt(11)with 9-amino acridine .reactions with DNA and study of their antiproliferative activity**.**
- **Tlegnov R.T (2008)** studied the Condensation of 9-aminoacridine with *p*-substituted aromatic aldehydes led to the synthesis of the corresponding azomethines



- **Kylene kehn-Hall *et al.,(*2009)** reported a 9-aminoacridine Inhibition of HIV-1 Tat Dependent Transcription.

- ***Sie-Tiong Ha1 et al.,*(2009)** reported a Synthesis and Mesogenic Properties of New Schiff Bases Comprising Benzothiazole Moiety.

- **Ruby Naaz *et al.,* (2010)** reported a water mediated condensation reaction of aldehydes and amines.



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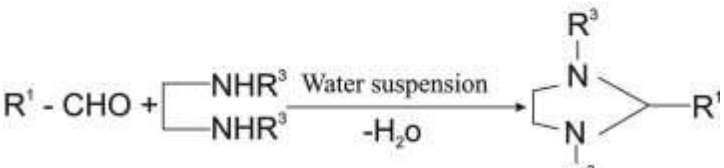
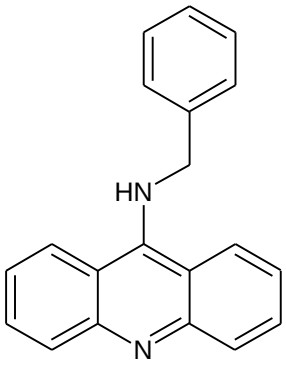


1 4 5

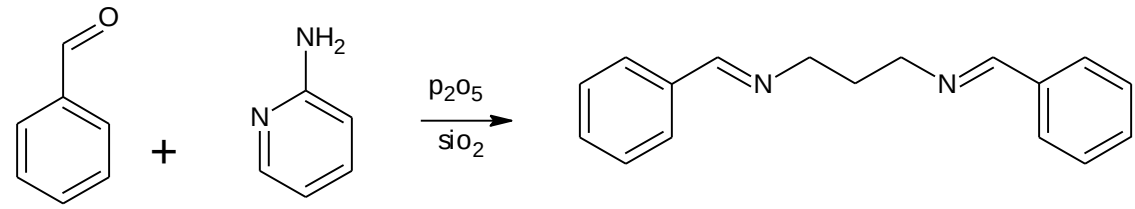
- **Gellerman Gary *et al.,* (2010)** "One-pot derivatization of medicinally important 9-aminoacridines by reductive amination and SNAr reaction"



- **Yan-Hua Cai *et al.*, (2010)** reported a Synthesis of Schiff Base Derived from *p*-Aminobenzoic Acid by Solvent-Free Reaction Using Jet Milling.

- **Shelar Mahendra Devidas *et al*., (2011)** reported a Novel One-Pot Synthesis of Schiff Base Compounds Derived from Different Diamine & Aromatic Aldehyde Catalyzed by P2O5/SiO2 under Free-Solvent Condition at Room Temperature

- **M. M. Murhekar et al., (2011)** Synthesis of Schiff bases by organic free solvent method.



.

- **Eman Turky Shamkhy *et al*., (2011)** reported a Preparation of New Schiff Base Derived from Cyclohexylamine with Piperonaldehyde and its Cu+2, Co+2 and Rh+3 Metal Complexes.

- **Muhammad Aqeel Ashraf, *et al.*, (2011)** reported a Synthesis, Characterization and Biological Activity of Schiff Bases

- **Tarek Aboul-Fadl *et al.*, (2011)** reported a Microwave-Assisted Solution-Phase Synthesis and DART-Mass Spectrometric Monitoring of a combinatorial Library of Indolin-2, 3-dione Schiff Bases with Potential Antimycobacterial Activity.

- **John Maria Xavier *et al*., (2012)** reported the Synthesis and spectral characterization of an aminoacetophenone-based Schiff base and its interaction studies with ascorbic acid. .

- **Savalia RV *et al.*, (2013)** reported a Rapid and Economic Synthesis of Schiff Base of Salicylaldehyde by Microwave Irradiation.

AIM & OBJECTIVE

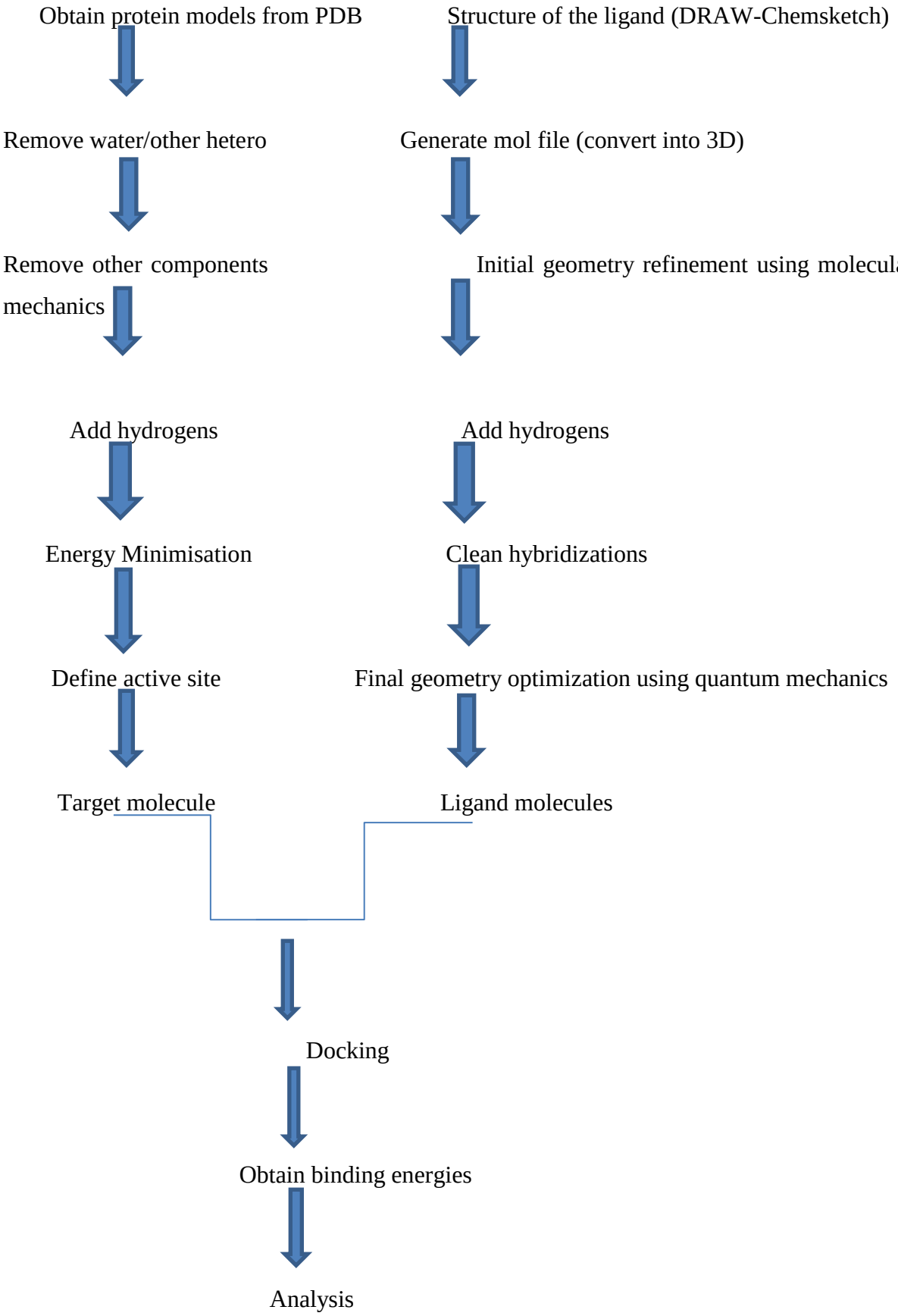
AIM

To develop novel and potent methoxy mycolic acid (mmaa2) 1TPY inhibitors with anti- tubercular –activity.

OBJECTIVE

- ✓ Identification of the common Pharmacophore responsible for the inhibition of mmaa2 using hip-hop molecule of catalyst software 4.11 from accelrys.
- ✓ Using scaffold hopping technique, generation of 10,000 scaffolds from the drug.
- ✓ Prediction of anti-tubercular activity for the designed using the hyporefine model and to identify novel and potent mmaa2 inhibitor using Lipinski rule of five.
- ✓ The potent mmaa2 inhibitors attained as results can be used as lead for drug development.
- ✓ Docking of the lead with various derivatives using glide software to the target 1TPY.
- ✓ The derivatives of the compounds from the lead molecule which has higher glide score value were synthesized.
- ✓ Justification of the purity of synthesized compounds using the method of melting point, thin layer chromatography.
- ✓ Characterization of the synthesized compounds by using IR spectroscopy, MASS spectroscopy and NMR spectroscopy.
- ✓ *In vitro* screening anti-tubercular activity of synthesized compounds.

The present study carried out based on the below flow chart.



MATERIALS AND METHODS

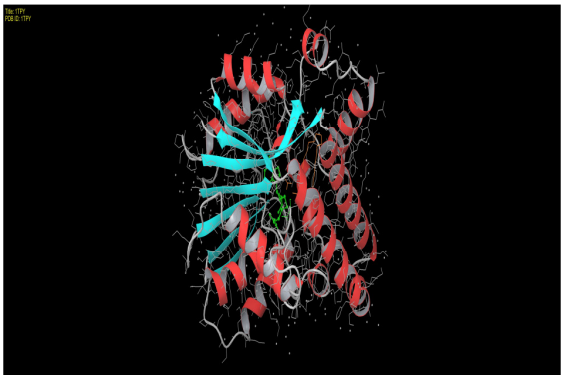
4.1) Drug design [20]

A process of design and discovery of new chemical entities using an automated docking program GLIDE (grid based ligand docking with energetics) maestro 9.0 Schrodinger suites. It searches molecules (ligands) having maximum favourable interactions with a receptor (target) usually a protein. Ligand is a single molecule whereas receptor may include proteins, metals and cofactors. It runs on rigid and flexible docking modes. The later generates conformations automatically for the input of each ligand and gives out the best fit pose of the molecule been docked on then receptor. The docking procedure involves protein preparation, ligand preparation, receptor grid generation and docking.

A.Protein preparation [41]

Protein preparation and refinement studies were performed on mmaA2. The protein was downloaded from the protein data bank with the following PDB ID: 1TPY, RESOLUTION 2.0A using protein preparation module (Schrodinger suite LLC) a typical PDB structure consists of heavy atoms, metal ions, cofactors, waters, etc. and can be multimeric. Protein preparation includes bond order adjustments, generating heterocyclic states, deleting water molecules except that co- ordinated to metals and waters which bridges ligand and protein are retained. Formal charges re-orient side chain hydroxyl groups and potential steric clashes via protein minimization with the OPLS-2005 force fields were assigned.

Fig.5



B) Ligand preparation:

Ligand preparation involves optimization of geometric parameters prior to docking .i.e. the compounds designed (structures) for docking should satisfy the following conditions.

- It must be dimensional (3D).
- IT must possess realistic bond lengths and bond angles.
- It must possess each of a single molecule having no covalent bonds to the receptor, covalent bonds, counter ions and fragments.
- It must possess all their hydrogens (filled vacancies).
- It must possess an appropriate protonation state for physiological pH values.
- The ligand preparation process comprises of a series of steps which includesation.
- Conversions, applying corrections and generating variations to the structures, there by rendering optimization.

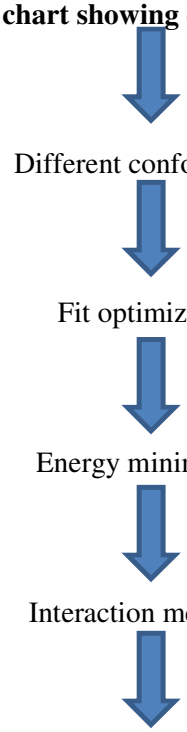
C) Receptor grid generation:

It involves generating a grid by representing the shape and properties of receptor using different tools of its wizard that progresses to the best fit pose of the ligand. This is done by defining the receptor structure by e excluding the crystal ligand already. The binding site in the grid was defined to a space of measuring 10A*10A*10A*box on the centre of crystal inhibitor. Receptor grid determines the size and position of active site. Setting up of glide constraints like position or attachment of the crystal ligand, hydrogen bond interactions and locating the hydrophobic contacts.

D) Docking procedure

Docking was carried out by browsing the ligand grid base name and receptor grid base name files from their output files, which contains the 3D structures of the synthesised compounds and the receptor grid generated from the prepared protein has the 3D structure of mmaA2 enzyme respectively. The G score of the compounds were picturised in XP visualizer under glide software.

Flow chart showing design &docking;



ENZYME PROFILE [28]

MmaA2 is required for introduction of the distal cyclopropane ring in the formation of meroacids. Analysis of a *mmaA2* deletion mutant of *M. tuberculosis* revealed that mycolic acid lacks a distal cyclopropane group and instead contains a *cis* unsaturation. Thus, *mmaA2* is required for the distal cyclopropane modification of _-mycolic acid

Functional category: lipid metabolism

E) DOCKING SCORE METHOD:

The compounds ranked by its G score which is the basic value obtained by the consideration of both rewards and penalties. Rewards- Lipophilic pair term and fraction of the total protein ligand vanderwaals energy, Hydrophobic enclosure reward, Hydrophobically packed Hydrogen bond, Hydrophobically packed correlated

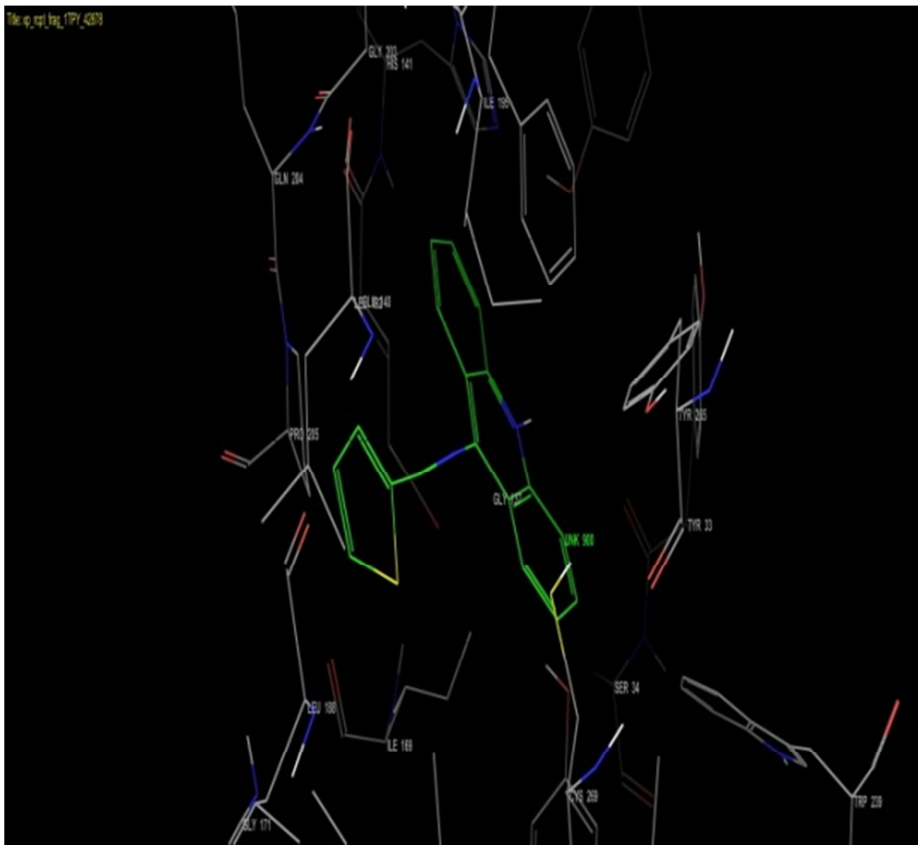
hydrogen bond, hydrogen bond pair term, Electrostatic rewards, sitemap ligand/ receptor, pi and cation, chlorine and bromine, low molecular weight these all are basic parameters consider as rewards of the docking score. Rewards values are noted in negative values.

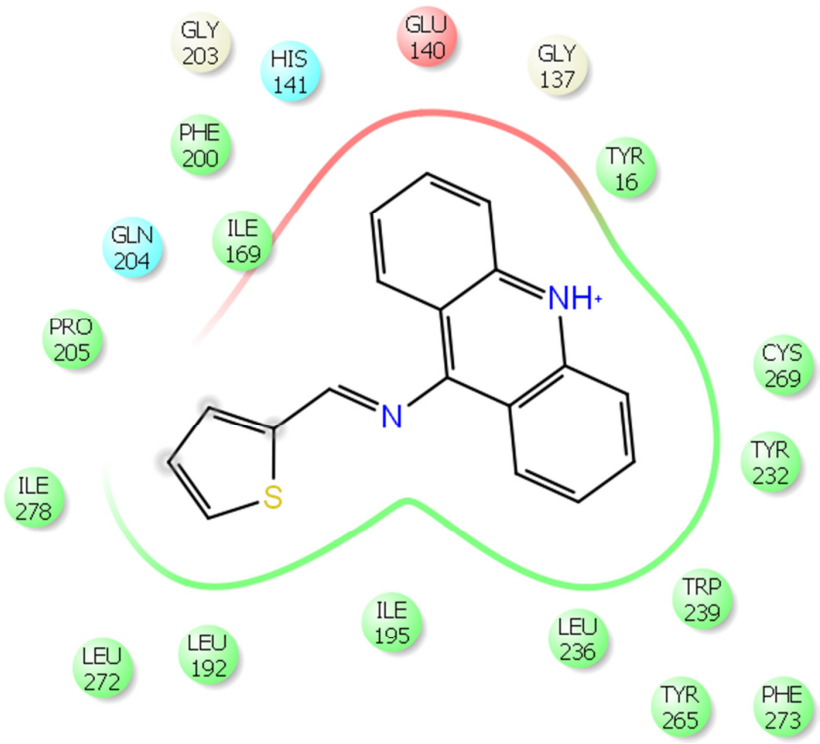
Penalty- Ligand with large hydrophobic contacts and low Hydrogen bond scores, Exposed Hydrophobic ligand groups, Rotatable bond, similarity these are the basic penalty parameters of the docking score. The penalty values should be less in values to indicate best compound. (Table.1, 2)

INTERACTIONS

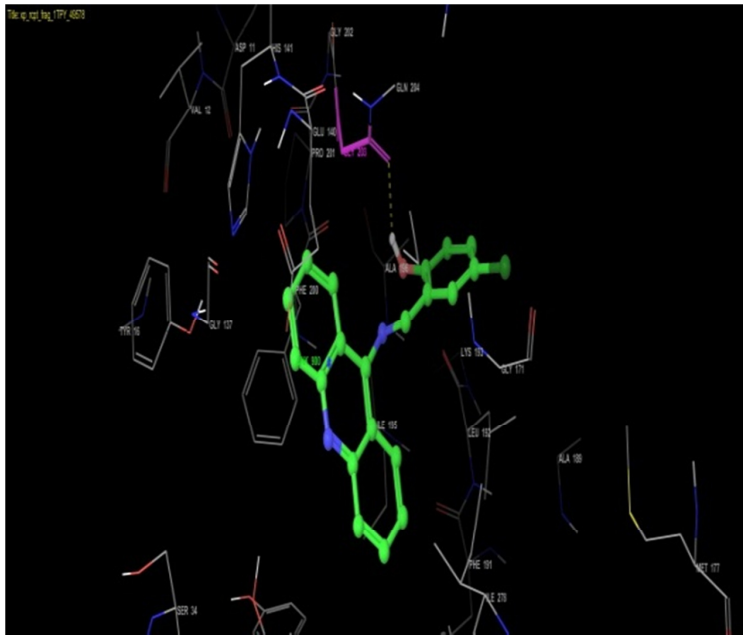
Binding mode of compound MI 3 and MI 4 in the active site of 1TPY dotted line indicates a hydrogen bond.

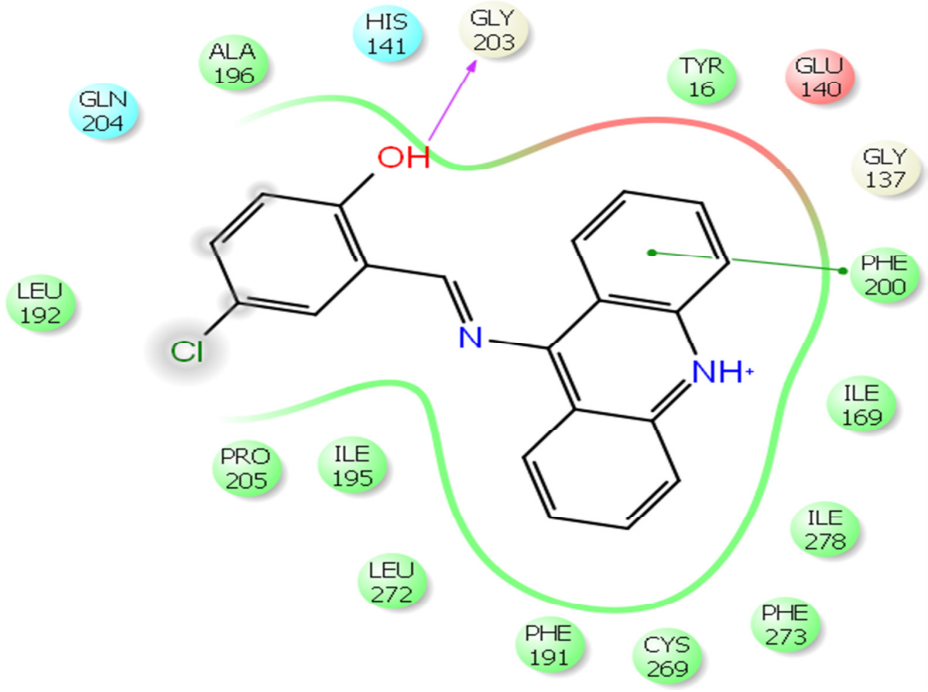
Interaction between MI 3 and 1TPY **Fig.6**





Interaction between MI 4 and 1TPY **Fig.7**





4.1.2 LIPINSKI'S RULE [24]

Lipinski's rule of five also known as the **Pfizer's rule of five** or simply the **Rule of five** (RO5) is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules.

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active.

REWARDS FOR MOLECULAS WITH 1TPY Table.1

Ligand	G score	Lipophilic Evdw	phobEn	PhobEn HB	PhobEN Pair HB	H Bond	Elect	Sitemap	Pi CAT	ClBr	LowMW
MI 3	-8.66	-5.25	-2.66	0	0	0	-0.14	-0.29	0	0	-0.5
MI 4	8.86	-5.97	-1.56	0	0	-0.61	-0.23	-0.25	0	0	-0.39

PENALITIES FOR MOLECULAS WITH 1TPY Table.2

Ligand	Penalties	HBPenal	PhobicPenal	RotPenal	Similarity
Mi 3	0	0	0	0.19	1
Mi 4	0	0	0	0.15	1

The rule is important to keep in mind during drug discovery when a pharmacologically active lead structure is optimized step-wise to increase the activity and selectivity of the compound as well as to insure drug-like physicochemical properties are maintained as described by Lipinski's rule. Candidate drugs that conform to the RO5 tend to have lower attrition rates during clinical trials and hence have an increased chance of reaching the market.

Lipinski's rule says that, an orally active drug has no more than one violation of the following criteria;

1. Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)

2. Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)

3. A molecular weight under 500 Daltons.

4. A partition coefficient log P less than 5.

Lipinski's Rule and Synthesized Compounds **Table.3**

Compound ID	**nON**	**nOHNH**	**MilogP**	**nrotb**	**TPSA**	**MOLECULAR FORMULA**	**MOLECULAR WEIGHT**
Mi 3	2	0	5.131	2	25.256	$C_{18}H_{12}N_2S$	288g/mol
Mi 4	3	1	5.358	2	45.484	$C_{20}H_{13}ClN_2O$	332g/mol

Log P -Partition coefficient

TPSA -Total Polar Surface Area

NOHNH -Number of hydrogen donors

nON -Number of hydrogen acceptors

4.1.3 Molinspiration

LogP (octanol/water partition coefficient) LogP is calculated by the methodology developed by Molinspiration as a sum of fragment based contributions and correction factors. Method is very robust and is able to process practically all organic and most organometallic molecules.

Milog P

Method for logP prediction developed at Molinspiration is based on group contributions. These have been obtained by fitting calculated logP with experimental logP for a training set more than twelve thousand, mostly drug-like molecules.

Molecular property predictions using MolSoft

In MolSoft all molecular property predictors are calculated using fragment-based contributions.

Refractivity A3

There are several potential classes of parameters used in QSAR studies. The selection of parameters is an important first step in any QSAR study. If the association between the parameter(s) than one of these rules may have problems with bioavailability.

Nrotb-Number of Rotatable Bonds

This simple topological parameter is a measure of molecular flexibility. It has been shown to be a very good descriptor of oral bioavailability of drugs.

Molecular Polar Surface Area PSA

Molecular polar surface area (PSA) is a very useful parameter for prediction of drug transport properties.

Molecular volume

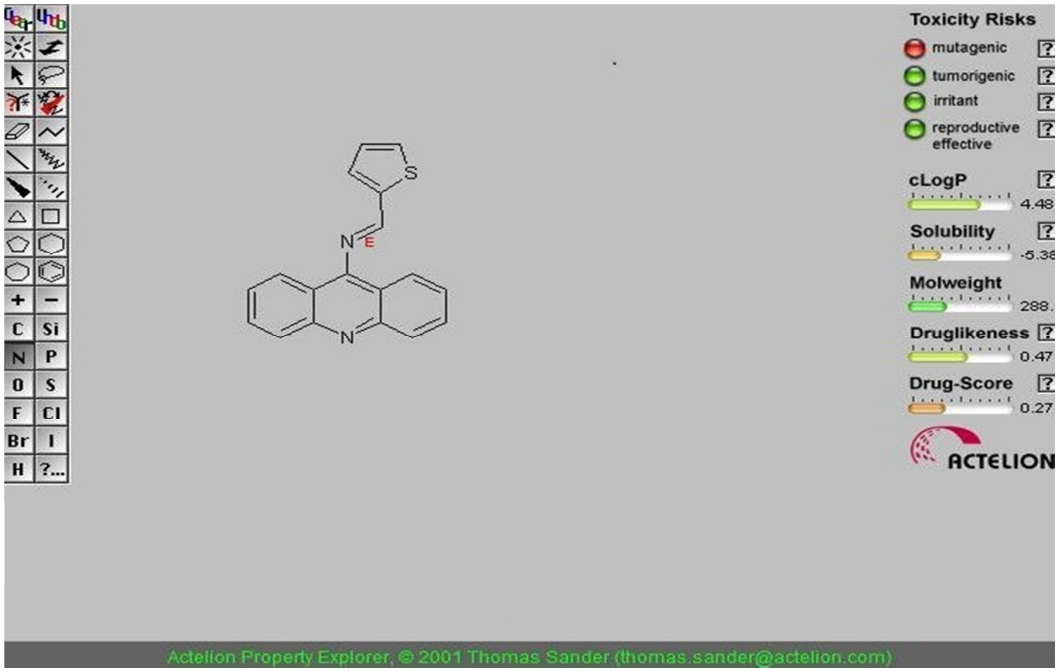
Molecular volume determines transport characteristics of molecules, such as intestinal absorption or blood-brain barrier penetration. Volume is therefore often used in QSAR studies to model molecular properties and biological activity.

4.1.4 ADMET Properties Predictions [17, 33,]

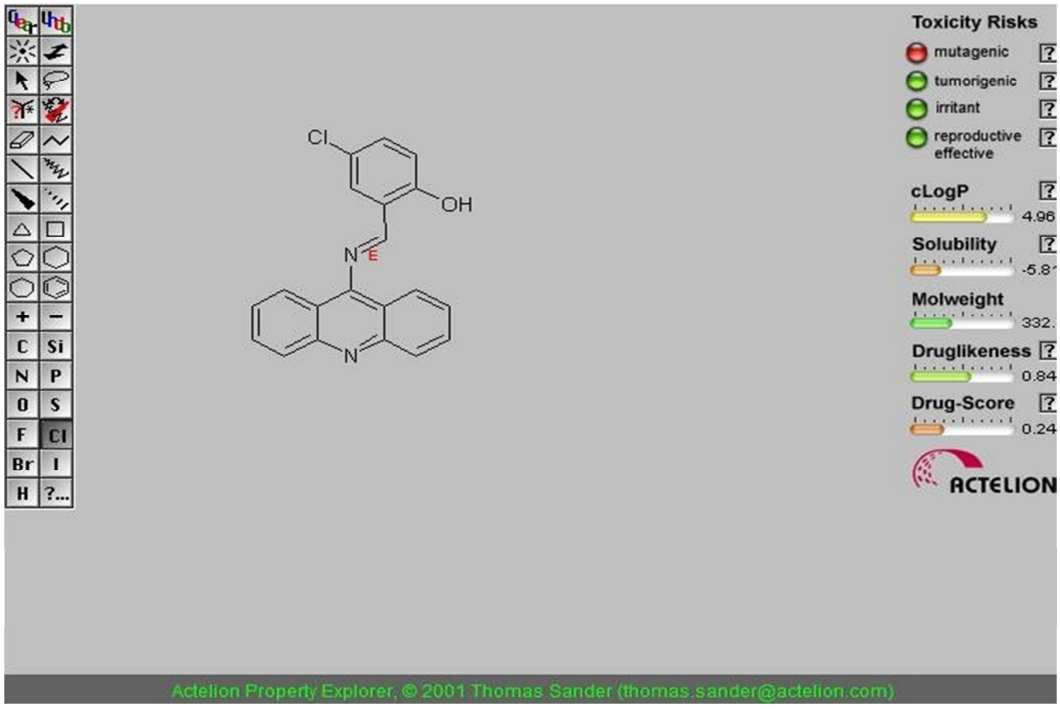
This was done using OSIRIS Property explorer which is an online tool. It lets one draw chemical structures and calculates on-the-fly various drug-relevant properties whenever a structure is valid. Properties with high risks of undesired effects like m1utagenicity or a poor intestinal

Absorption is shown in red. Whereas a green colour indicates drug conform behaviour. It determines water solubility of a substance (log S). More than 80% of the drugs on the market have estimated log S value greater than -4. It determines partition coefficient of a substance (c log P). c Log P is the logarithm of a compound's partition coeffient between n- octanol and water log (c octanol/c water). It has been shown for compounds to have a reasonable probability of being well absorbed their c log P value must not be greater than 5.0. Molecular weight, more than 80 % of all traded drugs have a molecular weight below 500 g/mol. Through this, the generated molecules were filtered out further by selecting only the molecules whose drug relevant properties conformed to those of most traded drugs.

OSIRIS RESULTS FOR MI 3 Fig.8



OSIRIS RESULTS FOR MI 4 Fig.9



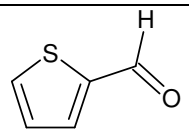
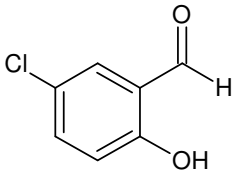
Toxicity Assessment of Compounds by Osiris Property Explorer Table.4

S.NO	Properties	MI 3	MI 4
1	Mutagenic	Red	Red
2	Tumorogenic	Green	Green
3	Irritant	Green	Green
4	Reproductive Effect	Green	Green

4.2. SYNTHESIS

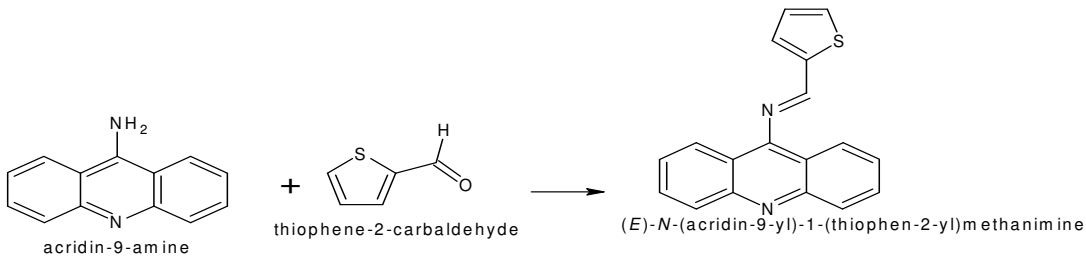
Synthetic scheme was framed for the hit compounds from docking and the procedure for synthesis was collected from literatures. The necessary chemicals of laboratory grade for the synthesis were procured from Sigma Aldrich and Synthesis was carried out.

4.2.1 REACTANT PROFILE [12] Table.5

Structure	Chemical Name	Molecular Formula	Formula Weight	Density	CAS. No
	thiophene-2-carbaldehyde	C_5H_4OS	112.1g/mol	1.2	98-03-3
	5-chloro-2-hydroxybenzaldehyde	$C_7H_5O_2Cl$	156.5g/mol	1.404	635-93-8

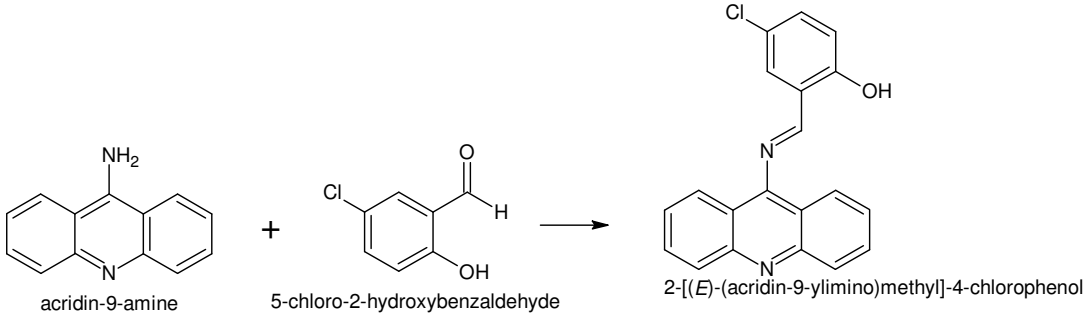
Scheme: 1 [6, 25, 38]

9 amino acridine and 2 Thiophene carboxaldehyde were weighed in the ratio of (1:1) and dissolved in methanol. The mixture was neutralised with sodium hydroxide. The pH was measure by pH paper .the reaction mixture was refluxed in a water bath for 3 hours. The reaction mixture was cooled to room temperature. Again the reaction mixture was neutralised with dilute hydrochloric acid. The precipitate was formed after the neutralisation then the precipitate was filtered and recrystallized from ethanol.



Scheme: 2

9 amino acridine and 5 Chlorosalicyldehyde were weighed in the ratio of (1:1) and dissolved in methanol. The mixture was neutralised with sodium hydroxide. The pH was measure by pH paper .the reaction mixture was refluxed in a water bath for 3 hours. The reaction mixture was cooled to room temperature. Again the reaction mixture was neutralised with dilute hydrochloric acid. The precipitate was formed then the neutralisation then the precipitate was filtered and recrystallized from ethanol.



acridin-9-amine + 5-chloro-2-hydroxybenzaldehyde → 2-[(*E*)-(acridin-9-ylimino)methyl]-4-chlorophenol

4.2.2 RECRYSTALLISATION

Ethanol was added to the synthesized compounds and heated until it dissolved completely. The clear solution thus obtained was filtered immediately and set aside for cooling .On cooling crystals gradually appeared.

4.2.3 METHODS FOR IDENTIFICATION

The synthesized compounds were identified by the following procedure,

1) Determination of melting point
2) Identification by TLC

MELTING POINT:

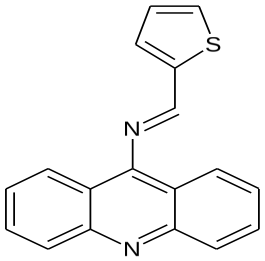
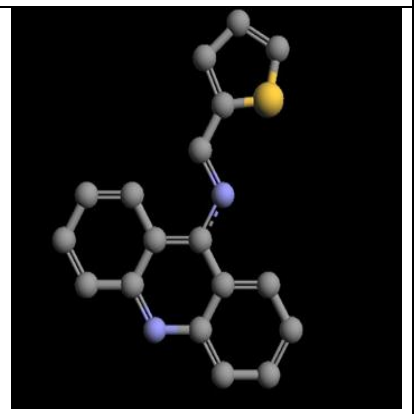
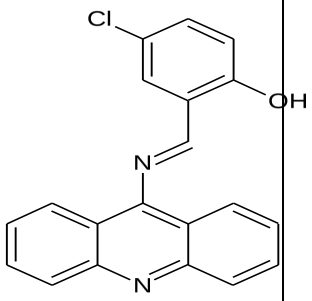
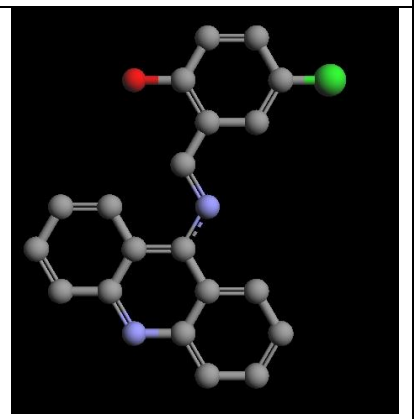
The melting points of the compounds were determined by capillary tube method.
The temperature at which the synthesized compounds started losing its crystallinity and changing from solid to liquid form were found and recorded. Care was taken that the compound did not undergo decomposition during the progress.

THIN LAYER CHROMATOGRAPHY

Precoated silica gel aluminium plates were used as stationary phase. The products and the reactants were made into solution in suitable solvents and spotted on the TLC plate. Elution of the spot with different mobile phase was tried out of which benzene Chloroform and ethanol in the ratio 5:4:1 was selected as most preferred one. The RF values were calculated and found that reactants and product have different RF value which confirms that reactants have formed the product.

Physical properties of the synthesized compounds and requirements and reaction conditions given the procedure. Products were obtained with a yield around 78% and then recrystallized. Then the physical properties such as appearance, solubility and melting point were determined and recorded. (Table.6)

PHYSICAL PROPERTIES OF SYNTHESIZED COMPOUNDS Table.6

S.No	COMPOUND ID	MOLECULAR WEIGHT	MOLECULAR STRUCTURE	SOLUBILITY	MELTING POINT(°C)	APPEARANCE	3D STRUCTURE
1	MI 3	288.3g/mol		**Methanol, dimethyl sulpoxide**	140-142	**Orange colour**	
2	MI 4	332.7g/mol		**Methanol, dimethyl sulpoxide**	126-128	**Black colour**	

4.2.4 CHARACTERIZATION [13, 18, 19]

The synthesized compounds were characterized by Infrared spectra (IR), Nuclear Magnetic Resonance spectra (1H NMR), and Mass spectra (MS).

INFRARED SPECTROSCOPY:

Infrared spectroscopy serves as the tool to ascertain the presence and absence of the functional group. In this technique, Sample was made into pellet with potassium bromide using pellet press and then the pellet was mounted on the pellet disc. Then the percentage transmittance in the IR frequency region was measured by running the spectrophotometer and the spectrum obtained was interpreted to find the result.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY:

Proton NMR spectroscopy helps us to study the number of equivalent protons and their environment thereby we can ascertain the structure of the molecules. By running NMR spectrophotometer, Chemical shift will be produced based on the nature and environment of the proton and this chemical shift provides information on the structure of the molecule. Thereby the structure of the synthesized chemical compound was elucidated. Table: 6 Multiplicity and relative intensities of resonance Signals from coupled groups of nuclei in saturated structures Number of adjacent nuclei Multiplicity of observed resonance Relative intensity of components multiplets.

Table.7

Number of adjacent nuclei	Multiplicity of observed resonance	Relative intensity of components multiplets
0	Singlet	1
1	Doublet	1 1
2	Triplet	1 2 1
3	Quartet	1 3 3 1
4	Quintet	1 4 6 4 1

Chemical shift data give a general indication of the ranges in _/ppm within which the resonances for different types of structure or functional groups will occur. The chemical shift range for protons that can be involved in hydrogen bonding is particularly wide, as the variation in degree of shielding of these protons may be

highly concentration-dependent Peak areas are measured by electronic integration of the resonance signals in a spectrum. For proton spectra, the total area, or integral, of a multiplet is directly proportional to the number of protons in the group. Integrals are recorded as a series of steps, generally displayed above each resonance signal. The vertical height of each step, in arbitrary units, gives the relative number of protons associated with the signal. The interpretation of proton spectra depends on three features: chemical shifts, multiplicities of resonances and integrated peak areas.

The following were the general approaches given to spectral interpretation, which was augmented by reference to chemical shift data, coupling constants and the spectra of known compounds.

- ✓ Noted the presence or absence of saturated structures, which gives resonances between 0 and 5 (trichloromethane at 7.25 is a notable exception).
- ✓ Noted the presence or absence of unsaturated structures in the region between about 5 and 9 (alkene protons between 5 and 7 and aromatic protons between 7 and 9). (Alkyne protons are an exception, appearing at about 1.5).
- ✓ Noted any very low field resonances (9 to 16) which are associated with Aldehyde and acidic protons, especially those involved in strong hydrogen bonding.
- ✓ Measured the integrals and calculated the numbers of protons in each resonance signal.
- ✓ Checked for spin-spin splitting patterns given by adjacent alkyl groups according to the *n+1* rule and Pascal's triangle. (N.B. The position of the lower field.
- ✓ Multiplet of the two is very sensitive to the proximity of electronegative elements and groups such as O, CO, COO, OH, Cl, Br, NH2, etc.)
- ✓ Examined the splitting pattern given by aromatic protons, which couple around the ring and are often complex due to second order effects.
- ✓ 1,4- and 1,2-disubstituted rings give complex but symmetrical looking patterns of peaks, whereas mono-, 1,3- and tri-substituted rings give more complex asymmetrical patterns

MASS SPECTROSCOPY:

The synthesized compounds were analysed with Mass spectrometer, which enables us to establish the molecular structure and molecular weight of the compound. In this Technique, compound under investigation was bombarded with a beam of electron producing ionic fragments. The relative abundance of the fragment ion formed depends upon the stability of the ion and the lost radical. The resulting charged species travel at different speed and path based on their mass to charge ratio to reach the detector and the recorded spectrum will have molecular ion peak, base peak and isotope peak to interpret structure of the synthesized compound.

Mass spectral data can be used to provide the following analytical information:

- An accurate relative molecular mass if the molecular ion can be identified
- An empirical molecular formula based on isotope peak intensities.

The interpretation of molecular mass spectra is accomplished by comparisons with the spectra of known compounds and the application of a set of empirical rules.

(i) The nitrogen rule states that compounds with an even-numbered relative Molecular mass must contain zero or an even number of nitrogen atoms, and those with an odd-numbered relative molecular mass must contain an odd number of nitrogen atoms.

(ii) The unsaturated sites rule provides a means of calculating the number of Double-bond equivalents in a molecule from the formula

No. of C atoms + 1⁄2 (no. of N atoms) - 1⁄2 (no. of H + halogen atoms) + 1

(iii) Double bonds and cyclic structures tend to stabilize the molecular ion, saturated rings losing side chains at the *a*-position.

(iv) Alkyl-substituted aromatic rings cleave at the *b*-bond to the ring giving a prominent peak at *m/z* 91, which corresponds to the tropylium ion, $C7H7^{+}$.

(v) Small neutral molecules, such as CO, C2H4, C2H2, H2O and NH3 are often lost during fragmentation.

(vi) The C-C bond adjacent to a heteroatom (N, O, S) is frequently cleaved leaving The charge on the fragment containing the heteroatom, whose nonbonding electrons Provide resonance stabilization.

(vii) McLafferty rearrangements in carbonyl compounds are common.

IR Spectrum of MI3 Fig.10

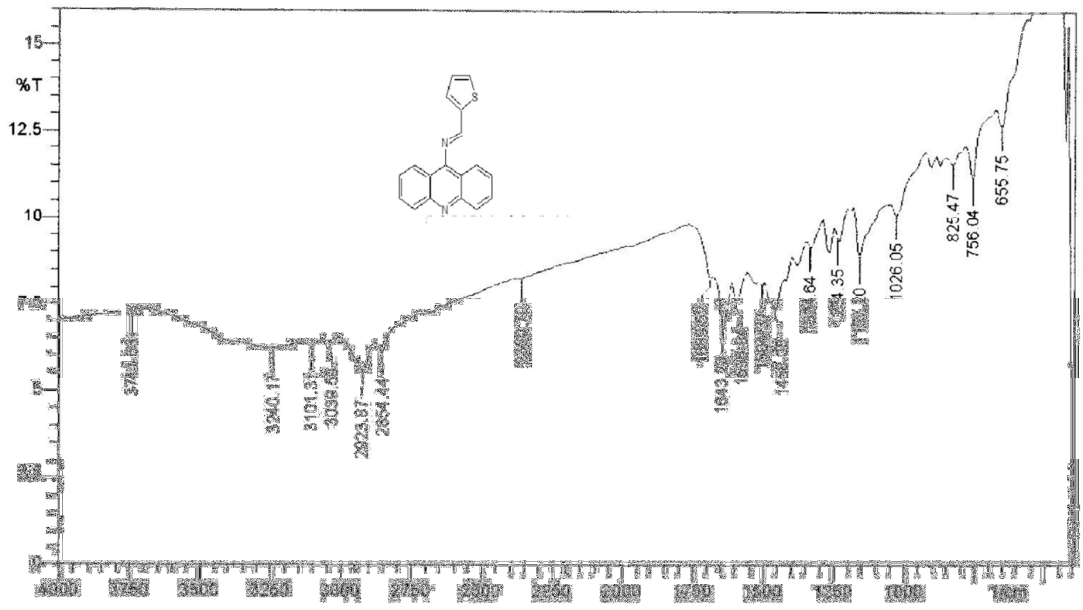


Table.8

COMPOUND NAME	IR REGION cm^{-1}
MI3	3039 (Ar C-H) 1643 (C=N) 1596(C=C) 756 (C-S)

NMR Spectrum of MI3 Fig.11

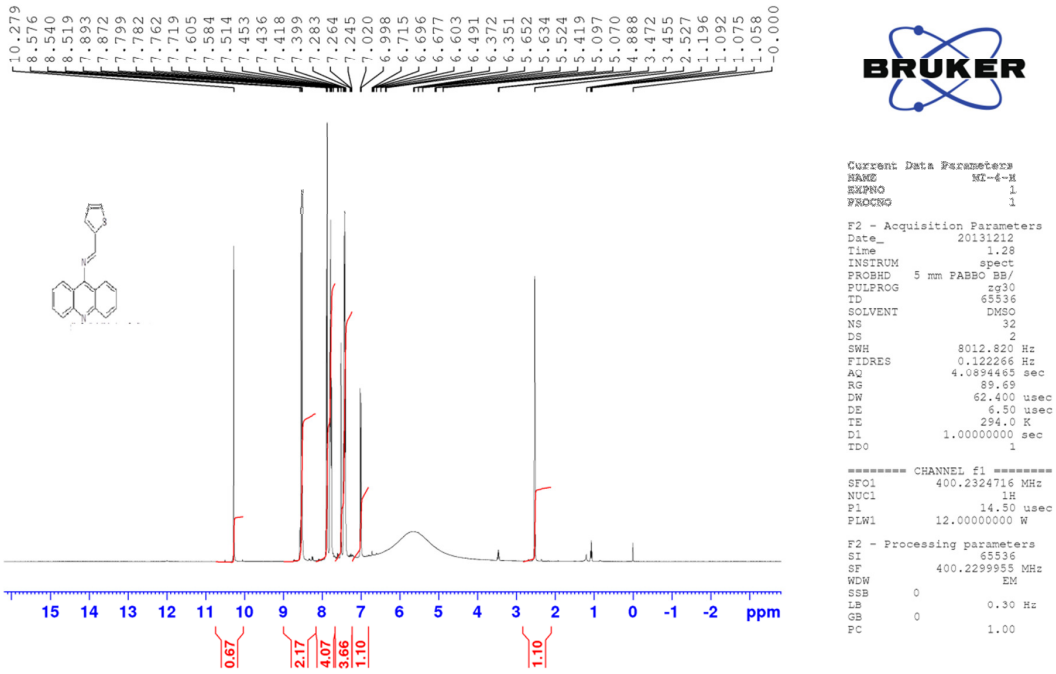


Table.9

COMPOUND NAME	1H NMR Data
MI3	9 8.62-8.7 (d, 3H, Ar-H). 7.88-7.99 (M, 5H, Ar-H). 7.42-7.56 (M, 3H, Ar-H). 2.46-2.63 (S, 1H, -CH).

MASS Spectrum MI3 Fig.12

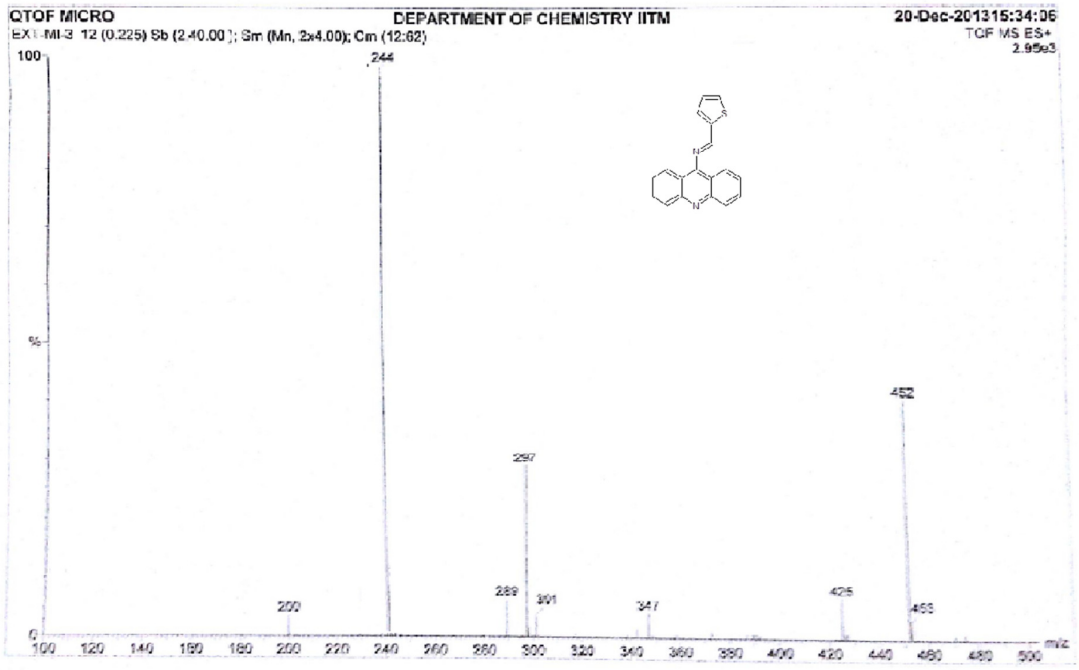


Table.10

COMPOUND NAME	Mol. for/ Mol Wt. (calculated)	m/e value (Relative abundance)
MI3	$C_{18}H_{12}N_2S$ /288	289 (M^+) 244 (B)

IR spectrum of MI4 Fig.13

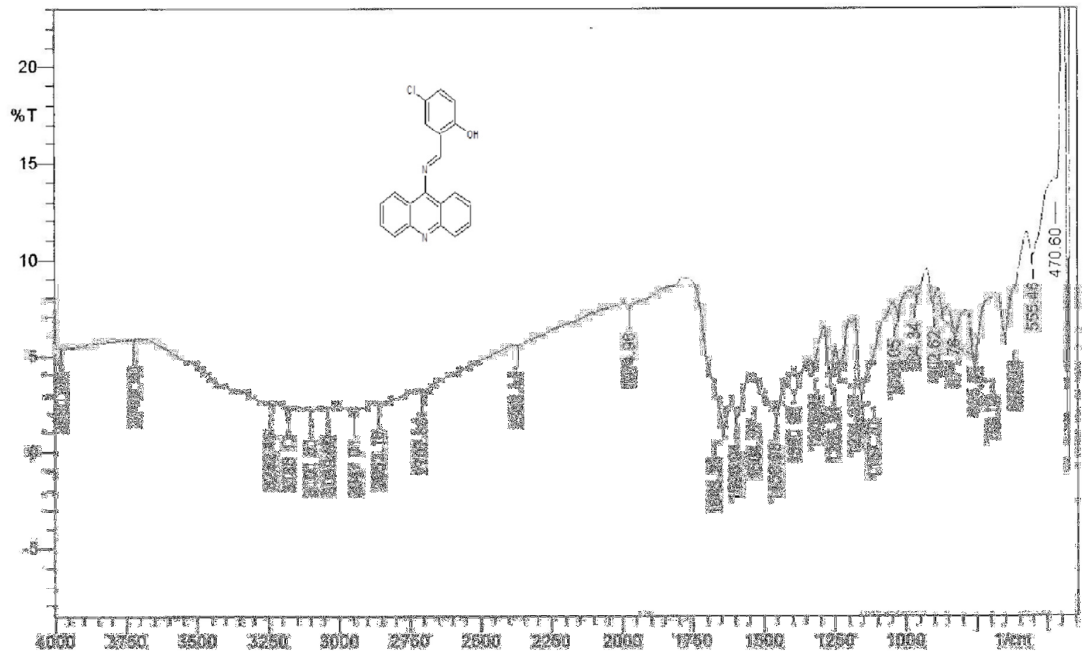


Table.11

COMPOUND NAME	IR REGION cm^{-1}
MI4	3232 (Ar O-H) 3039 (Ar C-H) 1643 (C=N) 1596(C=C) 750 (C-Cl)

NMR Spectrum of MI4 Fig.14

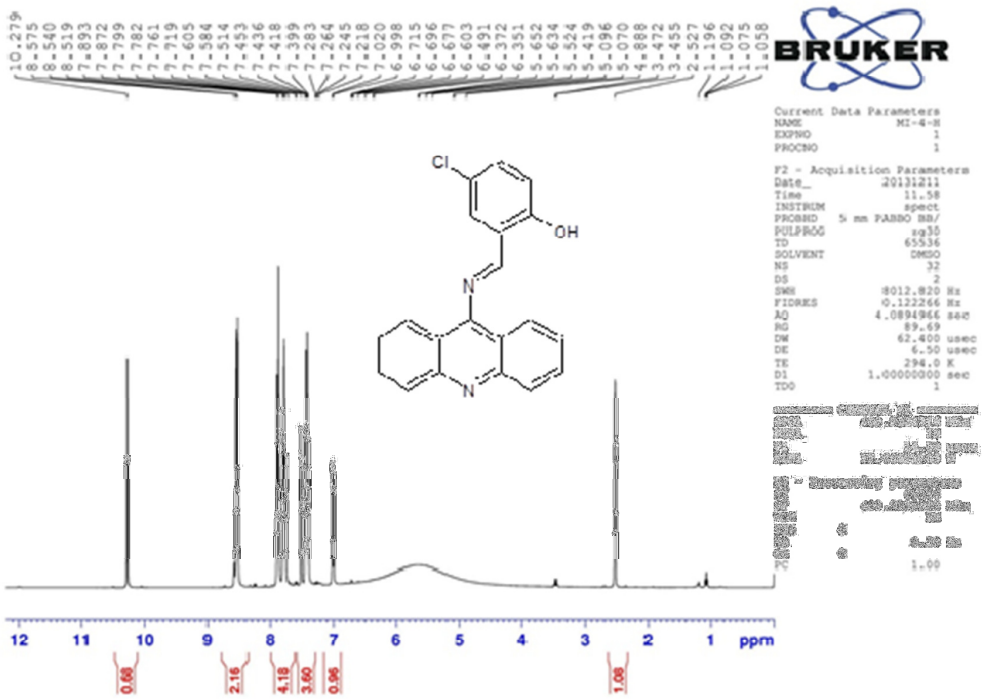


Table.12

COMPOUND NAME	1H NMR Data
MI 4	10.1-10.4 (S, 1H, OH) 8.3-8.7 (d, 2, Ar-H) 7.6-7.9 (m, 4H, Ar-H) 7.2-7.5 (m, 4H, Ar-H) 6.8-7.1 (d, 1H, Ar-H) 2.3-2.6 (S, 1H, CH)

MASS Spectrum of MI4 Fig.15

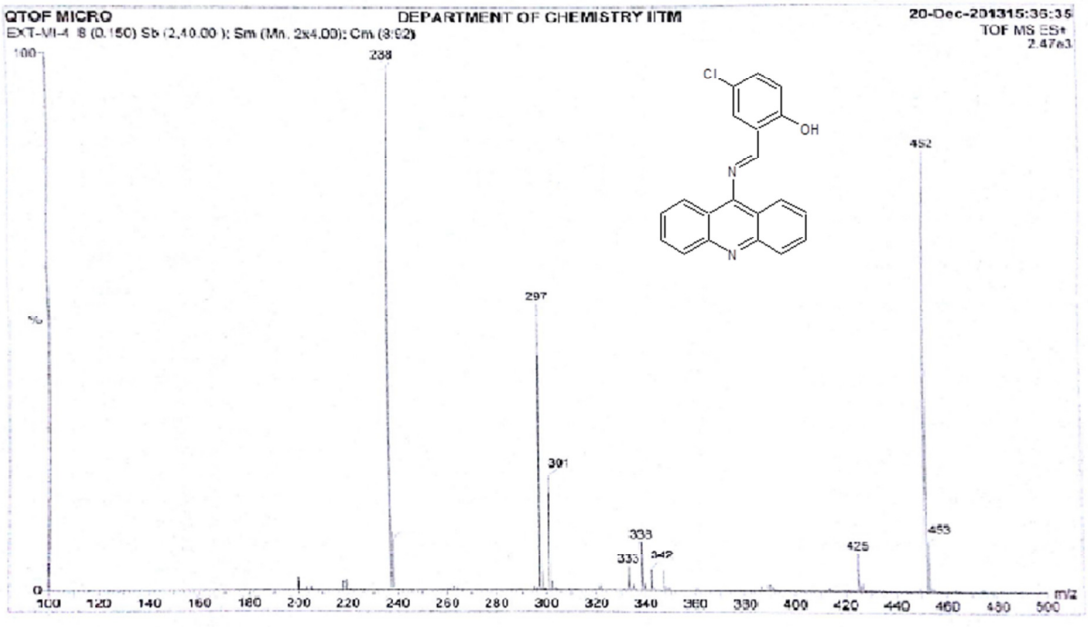


Table.13

COMPOUND NAME	Mol. for/ Mol Wt. (calculated)	m/e value (Relative abundance)
MI4	$C_{20}H_{13}N_2OCl$ / 332	333 (M^+) 238 (B)

4.3) BIOLOGICAL SCREENING

4.3.1ANTI- TUBERCULAR ACTIVITY [4, 27, 34,]

As discussed, from earlier to the present status, tuberculosis is existing with (extreme &multi-drug resistance), increased susceptibility in transmission, their complex pathophysiology leading to higher risks of infections to other organs and increases the rate of mortality.so essentially there is an urgent and rapid need of drugs to combat this disease, which in turn it is screened for anti-tuberculosis activity.

There are various high through put assays are available for screening of new chemical entities against tuberculosis of which ,we have chosen MABA assay and the rest of us are enlisted below.

- Micro plate Alamar blue assay (MABA)
- BACTEC assay
- Lactiferous reporter phage assay (LRP)
- REMA assay
- Broth dilution assay
- Middle brook (7H 9, 7H 10, 7H 11,) Agar dilution assay

Principle

The micro plate Alomar blue assay (MABA) is an indirect colorimetric DST method for determining the MICs of TB drugs for strains of mycobacterium tuberculosis .in this assay, the redox indicator Alomar blue monitors the reducing environment of the living cell.it turns from blue to pink in the presence of mycobacterial growth.

Procedure

The anti- mycobacterial activity of compounds (MI3, MI4) were assessed against *M.tuberculosis* using micro plate Almar blue assay (MABA)

This methodology is non –toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

Briefly, 200ml of sterile de-ionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.

The 96 wells plate received 100ml of the middle brook 7H9 broth and serial dilution of compounds was made directly on plate.

The final drug concentrations tested were 100 to 0.8mg/ml

Plates were covered and sealed with parafilm and incubated at 37c for dive days.

After this time 25ml of freshly prepared 1; 1 mixture of almar blue reagent and 10% tween 80 was added to the plate and incubated for 24 hours.

A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth.

The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

Advantages:

- It has accurate time –course measurements,
- It has high sensitivity and linearity,
- It involves no cell lies,
- It involves no cell lysis,
- It is ideal for use with post –measurement functional assays,
- It is flexible as it can be used with different cell models,
- It is scable and can be used with fluorescence- and/or absorbance –based instrumentation platforms,
- Finally ,it is non-toxic, on –radioactive and is safe for the user
- Application
- Especially meant for studies on mycobacterium tuberculosis
- Used extensively in cell viability and cytotoxicity studies

Other applications

- Exploited for monitoring immune cell proliferation and function.
- Useful in identifying resistance-compromised mutants and identification of compounds with anti-biofilm activity.
- Recently, the Almar Blue assay has been used to study fungicide sensitivity of five plant pathogens, Monilina fructicola, Botrytis cinera, Verticullium dahlia, Colletotrichum spp.and Alternaria alternate.
- Alamar Blue assay to evaluate plant cell viability and /or proliferation in tomato cell suspension was studied

RESULT AND DISCUSSION

5.1 DRUG DESIGN

5.1.1 Glide Docking

A promising heterocyclic nucleus Schiff base Which was Prominently proved of exhibiting different biological activities had been used to prepare a database which was then docked against 1 TPY protein for Antitubercular activity.by using Glide Software (Maestro 9.1) Compound MI 4gave favourable interaction with the active site amino acids Gly 203 and Phe 200

Extra Precision (XP) scoring function was utilised to rank order compounds. The different derivatives of Schiff base were docked to the specific protein target site. The best compounds with top scored were filtered. Top scored compounds were selected and synthesized.

5.1.2 Lipinski Rule and Toxicity Risk Assessment

All the compounds pass the Lipinski rule there is no violation in the basic properties and the ADME/Toxicity profile were studied. It's proves that our molecule has ability to reach the target site for the action.

5.2 SYNTHESIS &CHARACTERIZATION

5.2.1 Synthesis

The compound mi 3-mi 4 prepared by the condensation of primary amine with aromatic aldehyde

5.2.2 Characterization

The synthesized compounds were recrystallized and the reaction was monitored by TLC. The melting point of the products were found and are presented uncorrected. The characterization was carried out using sophisticated instruments like, IR, NMR, and MASS Spectroscopy.

The results are shown below:

IR SPECTROSCOPY

The IR Spectroscopy has been used to identify the synthesized compounds. The presence of impurities like starting raw material i.e. aldehyde, amine were ruled out by ensuring absence of the functional group of the parent compounds.

The synthesized compounds show the following specific characteristics stretching vibrations.

Compound MI 3

3039cm^{-1}- Aromatic C-H Stretching

1643 cm^{-1} – C=N Stretching

1596 cm^{-1} -C=C Stretching

756 cm^{-1} - C-S Stretching

Compound MI 4

3232cm^{-1}- Aromatic O-H Stretching

3039cm^{-1}- Aromatic C-H Stretching

1643cm^{-1} – C=N Stretching

1596cm^{-1}-C=C Stretching

750cm^{-1} - C-Cl Stretching

The presence of the Schiff base functional and the absence of reactant functional groups confirmed our compound has formed.

NMR SPECTROSCOPY

The H1 NMR Spectral data of all the synthesized compounds were in conformity with the structural assigned. The H1NMR Spectral data of all the synthesized compounds were in conformity with the structure assigned. A short sharp singlet was observed at 4.9- 5.0 for all compounds which might be due to the presence of NH proton.

Compound MI 3

9 8.62-8.7 (d, 3H, Ar-H).

7.88-7.99 (M, 5H, Ar-H).

7.42-7.56 (M, 3H, Ar-H).

2.46-2.63 (S, 1H, -CH).

Compound MI 4

10.1-10.4 (S, 1H, OH)

8.3-8.7 (d, 2, Ar-H)

7.6-7.9 (M, 4H, Ar-H)

7.2-7.5 (M, 4H, Ar-H)

6.8-7.1 (d, 1H, Ar-H)

2.3-2.6 (S, 1H, CH)

MASS SPECTROSCOPY

All the synthesized compounds exhibited molecular ion peak (M^+) of varying intensities ascertaining the molecular weights of the compounds. It was observed that the compounds. It was observed that the compounds MI 3, MI 4 gave fragment ions at m/e ratio values 289, 333.

All the above spectral data confirmed that assigned structure of synthesized compounds.

5.3 BIOLOGICAL SCREENING

Antitubercular Screening

The antimycobacterial activities of the synthesized compounds were determined by MABA method. The organism used in this study is M.tuberculosis H37Rv.

All the synthesised compounds showed antibacterial activity to varying degree against the organism tested. The pathogen tested was susceptible to all the synthesized compounds but the minimum inhibitory concentration(MIC) for the compounds varied between 100 to 0.2 µg/lathe data pertaining to these observations are presented in no. and the growth of organisms is shown in Fig no. It was observed from the study that minimum inhibitory concentration of the synthesized showed good antimycobacterial activity. Inhibition was compared using standard pyrazinamide 3.125µg/ml ciprofloxacin 3.125µg/ml, and streptomycin 6.25 µg/ml as standard.

Among the synthesized compounds MI 2, MI 5 show good activity at minimum concentration 3.12 µg/ml. Compounds MI 1,MI 3,MI 4, exhibit moderate activity minimum inhibition at 6.25 2 µg/ml.

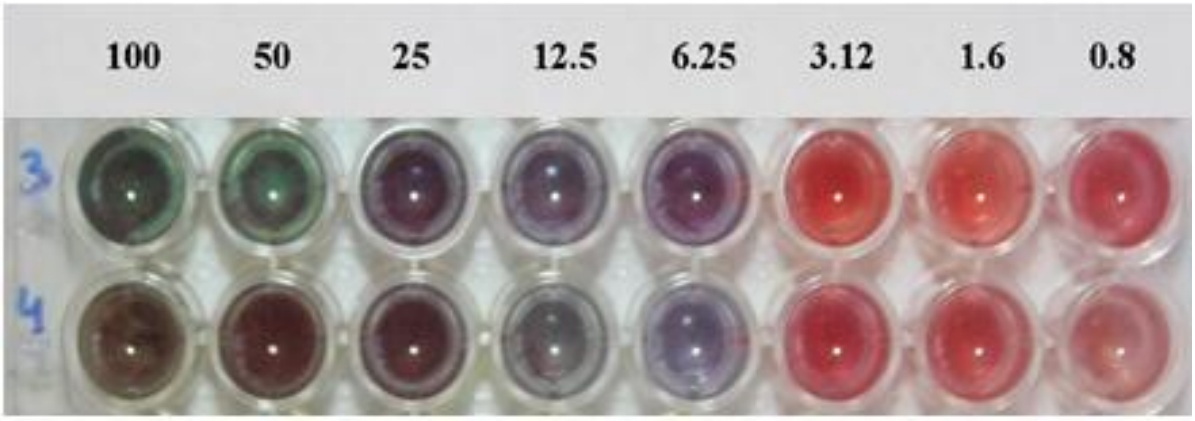
5.3.1 Anti-TB activity using Almar Blue Dye

1) The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using micro plate Almar Blue assay (MABA).
2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.
3) Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.
4) The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilution of compounds was made directly on plate.
5) The final drug concentrations tested were 100 to 0.2 µg/ml.
6) Plates were covered and sealed with parafilm and incubated at 37ºC for five days.
7) After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.

8) A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth.
9) The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

Inhibition of synthesized compounds against Mycobacterium Tuberculosis at Lowest concentration.

Fig.16



Organism - M.tuberculosis H37Rv

B-Blue indicates sensitive (S)

P-Pink indicates resistant (R)

Table.14

SI. No	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	MI3	S	S	S	S	S	R	R	R
2	MI4	S	S	S	S	S	R	R	R

ANALYSIS OF G SCORE VS ACTIVITY OF SYNTHESISED MOLECULES

Table.15

SI NO	COMPOUND ID	G SCORE	ACTIVITY
1	MI 4	-8.86	6.25
3	MI 3	-8.66	6.25

ANALYSIS OF G SCORE VS ACTIVITY OF STANDARD MOLECULES

Table.16

SI NO	COMPOUND ID	G SCORE	ACTIVITY
1	Ciprofloxacin	-7.5	3.15
2	Pyrazinamide	-5.8	3.15
3	Streptomycin	-4.9	6.25

.

SUMMARY AND CONCLUSION

1. 1TPY a critical enzyme for the growth of Mycobacterium tuberculosis was chosen for our study after review of literature.

2. A database of 50 molecules with high prospect of inhibiting the target 1TPY were carefully chosen by making changes into the known hit molecule, here the acridine nucleus.

3. Candidate molecules were designed and docked against 1TPY protein using Glide® (grid based ligand docking with energetics) program.

4. Two molecules with good G-Score (high stablility/lower binding energy) were shortlisted for the synthesis . They were labelled as MI3, and MI4

5. Two compounds were synthesized with good yield by conventional Schiff base reaction involving condensation between the 9-Amino Acridine and different types of aromatic aldehydes.

6. The purity of the synthesized compounds were evaluated by melting point, TLC, recrystallization.

7. The synthesized compounds were characterized by IR spectroscopy, NMR spectroscopy and Mass spectroscopy.

8. The pure compounds were screened for In-vitro Anti-tubercular activity by Microplate Alamar Blue Assay (MABA) method.
9. Two compounds showed significant *invitro* antitubercular activity. The compounds MI3, MI4, showed minimum inhibitory concentration at 6.25mg/ml against the MIC of standard drugs Pyrazinamide: 3.125mcg/ml, Ciprofloxacin: 3.125 mcg/ml, Streptomycin: 6.25 mcg/ml.

Conclusion:

The G-Score of the synthesized compounds ranged between -8.86 to -8.66.

The docking scores of Standard drugs against 1TPY were
Pyrazinamide: -5.8 kcal/mol,
Ciprofloxacin: -7.5 kcal/mol,
Streptomycin: -4.9 kcal/mol.
So there is correlation between the docking study and In-vitro Antimycobacterial activity. The results of our study conclude that 1TPY is a critical enzyme for Antimycobacterial activity. These compounds can be attractive starting point to find newer molecules by fine tuning the structures to yield compounds with better activity against Mycobacterium tuberculosis.

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